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MODERN IDEAS ON SPONTANEOUS GENERATION

BY

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Conference Chairman and Consulting Editor

ROSS F. NIGRELLI

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INTRODUCTORY REMARKS

By Harold F. Blum

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The title of this monograph probably has raised strong doubts in the minds of many of its readers at the same time that it caught their imaginations. The term "spontaneous generation" was taboo among biologists only a few years ago. Indeed, since Lazzaro Spallanzani's time, and certainly since that of Louis Pasteur, the idea that life does not spring from the nonliving environment, but comes only from existing life, has been staunchly accepted. However, our concepts of evolution and, particularly, the biochemical evidence seem to require that at some time in the distant past life did emerge from a nonliving milieu. It is with problems having to do with such emergence that we are concerned.

Whether we refer to the event as the origin of life or as spontaneous generation is, perhaps, of little importance, as long as we agree upon what subject we are discussing. On the other hand, however, the careless use of words can lead to careless thinking, and the term "spontaneous," like many others, may have different implications in different contexts. The contributors to this publication represent a variety of disciplines in each of which the connotation given to certain terms and, indeed, the whole methodology of approach may be quite different in some instances. Unless we bring a certain tolerance to such a discussion—a desire to understand the other man's argument even though he may attach somewhat different meanings to words—we are likely to end in a confusion of tongues. Consequently, I shall devote a little space to matters of terminology in an attempt to place the problem with which we are concerned into proper perspective.

I do not have the temerity to propose a complete definition of life, but I suggest that the term be applied only to the systems of existing species of plants and animals and their ancestors going back to their emergence from nonliving matter. This strict definition of the term imposes restrictions on our thinking because it confines us to the origin of the kinds of systems whence the existing species could have evolved by natural selection. However, even this rigid limitation includes not only systems that could replicate themselves, but those that could mutate in their pattern of replication so as to provide new species for selection. Eliminated at once are many "autocatalytic" or cyclic systems that we might imagine to have existed or now to exist elsewhere on some other planet; perhaps they did exist briefly on this one, but did not join the main stream of evolution since they could not serve as ancestors of the living things we know.

My use of the term "natural selection" is, I think, compatible with modern Darwinian concepts. This usage does not include those nonreplicating processes, governed by thermodynamic and kinetic factors, by which certain chemical compounds become predominant among the products of a reaction

mixture to the exclusion of others. It seems necessary to believe that the latter kind of selection of chemical compounds, which might be distinguished by the term "chemical selection," had a dominant evolutionary role before the advent of our replicating ancestors, since it determined the kind of chemical species from which the earliest of those ancestors could have been built, and hence placed limits within which the subsequent stream of evolution has been forced to remain.[1, 2] Consequently, I do not wish to belittle either of these important evolutionary mechanisms, but I do wish to point out that they are functionally distinct and should not be confused. To this end I suggest that the term natural selection be applied only in its modern Darwinian sense, in which it enjoys priority of usage.

If we follow the "Ariadne's thread" of biochemical evolution—I use the apt metaphor of Marcel Florkin—we find not only that the types of chemical compounds found in living organisms support the idea of a common ancestry, as though the present multiplicity had stemmed from a relatively small number of types, but that certain species of compounds are present universally. Outstanding among these substances are proteins, nucleic acids, and materials concerned in the transfer of energy-rich phosphate groups, of which ATP (adenosine triphosphate) is the most common. From the universal occurrence of these three types of compound and the essential roles they play, we are constrained to think that they were present in the earliest ancestral replicating systems, if we accept the definition of these systems that I have proposed. Each of the three plays an essential role in at least one of three functional properties of replication which, in a sense, constitute an irreducible minimum. These properties I describe[3] as follows:

(1) *Energetic.* These properties have to do with the supply and utilization of free energy to do the necessary work for replication, including chemical syntheses. ATP is particularly associated with this process.

(2) *Kinetic or catalytic.* These properties concern the modulation of the rates of reactions concerned in the energetic function and all others. The proteins play their irreplaceable role as enzymes in this area.

(3) *Spatial.* This consideration has to do with the accurate reduplication that gives stability to species and the modification of pattern that constitutes mutation. Here the nucleic acids play their great role, probably assisted by the proteins.

While these factors are separately describable in physical terms, their inseparable association in biological systems confers on the latter a degree of coordinated complexity that is not found in the physical world outside them. It is the origin of this type of complex organization that faces us when we approach the problem of the origin of life. I hope that we may gain some ideas about how these basic substances came to play their roles in the process of replication, without which there would be no life and no evolution of life. Perhaps it is still too early to approach this phase of the problem; however, I think its solution is a goal that we should keep always before us as we struggle with the problem of the origin of life.

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THE FORMATION OF ORGANIC COMPOUNDS ON THE PRIMITIVE EARTH

By Stanley L. Miller

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Introduction

One of the most fundamental problems of biology is posed by the question "How did life arise on the earth?" The theory of evolution offers an explanation for the development of complex multicellular living organisms from unicellular organisms, but this theory does not explain the development of the first organism. To assume that life arose from inorganic matter presents overwhelming difficulties. One must assume, not only that a self-duplicating organism could be made from inorganic matter, but that the organism could contain the complex apparatus needed to synthesize all of its components and energy requirements from carbon dioxide, water, and light.

In his celebrated book *The Origin of Life* (1938), Oparin proposed that spontaneous generation of life would be facilitated if the ocean contained a large amount of complex organic compounds similar to those present in living organisms. These compounds would serve both as structural components and as the energy supply for the first organisms. Oparin also suggested that the earth had a reducing atmosphere of methane, ammonia, water, and hydrogen in its early stages, and that organic compounds might be formed under these conditions.

Urey (1952a, 1952b), in considering the problem of the formation of the solar system, also proposed that our planet originally had an atmosphere of methane, ammonia, water, and hydrogen. Unlike Oparin's qualitative arguments, Urey's reasoning was based on thermodynamics. Methane and ammonia are the thermodynamically stable species of carbon and nitrogen in the presence of excess hydrogen.

Experimental support for these theories has come from studies of the action of electrical discharges on these gases (Miller, 1953, 1955, and 1957). These results will be summarized, and some of their implications will be discussed.

As a basis for discussion, the following model of the earth in its early stage is proposed. The atmosphere was reducing, and the oceans covered an appreciable fraction of the surface of the earth. The temperature is assumed to have been less than 100° C. The sources of energy for the production of the initial organic compounds were ultraviolet light, electrical discharge, and high temperatures (under local conditions such as those produced by volcanoes). Although the level of radioactivity was higher than at present, the energy available was still quite small, and there is no evidence that the intensity of cosmic rays was ever sufficient to compare with the energy from the sun.

The energy from ultraviolet light probably would be greater than that from electrical discharges. Because of the difficulties of working with ultraviolet light in the region in which these reduced gases would absorb (<2000 Å.), I decided to work with electrical discharges.

Spark Discharge: Run 1

An approximation of the proposed model is shown in FIGURE 1. The apparatus is made of Pyrex, with tungsten electrodes. The water in the small flask is boiled to promote circulation and to bring water to the region of the spark. The products of the discharge are condensed and flow through the U-tube, which prevents circulation in the wrong direction. The nonvolatile

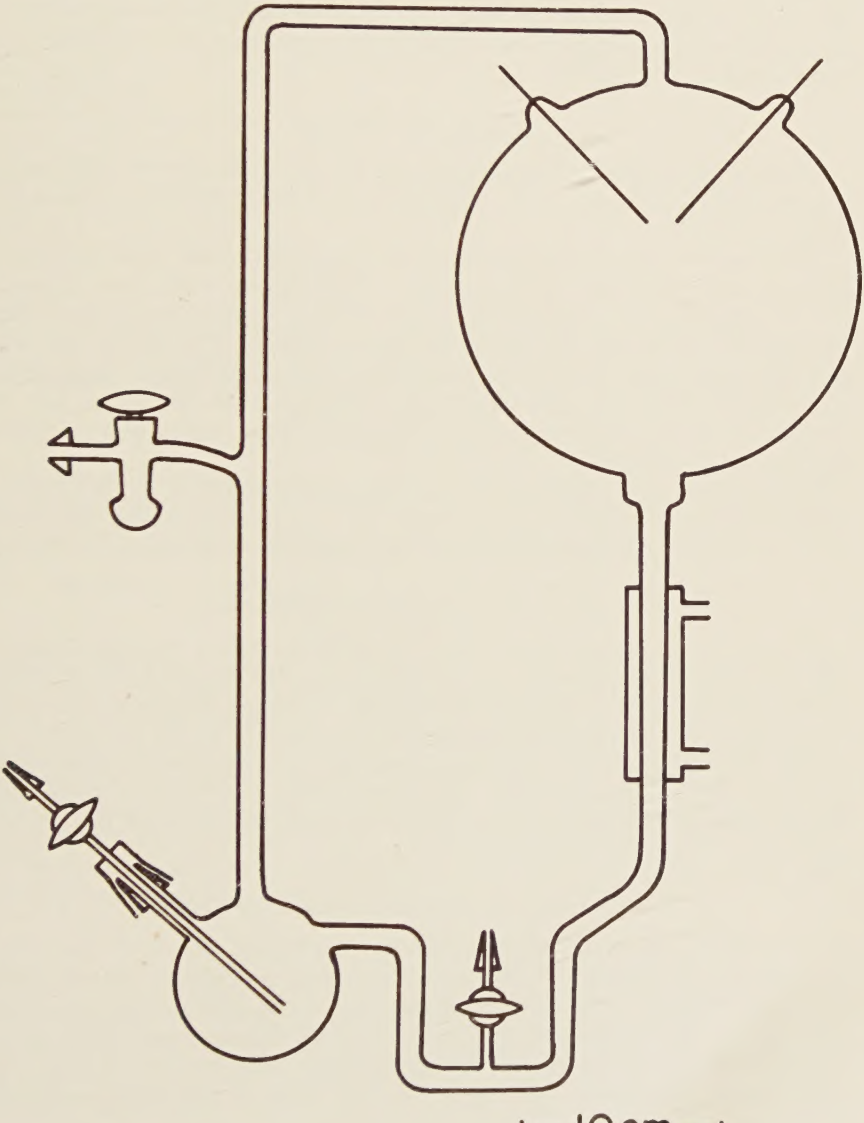

Figure 1. Spark-discharge apparatus.

compounds accumulate in the small flask. The spark discharge is produced by a high-frequency Tesla coil that has a peak of 60,000 volts.

The pressures of hydrogen, methane, and ammonia were 10, 20, and 20 cm. of Hg, respectively. The water was boiled, and the spark was operated continuously during a run. Colloidal silica that originated from the action of ammonia on glass, together with a yellow polymer, formed in the boiling flask during the run. The yellow polymers have a strong ultraviolet absorption, but have no peaks above 230 mμ. The nondialyzable compounds were hydrolyzed and chromatographed. Only a very small amount of amino acid was present in this fraction.

Analysis of Products

Analysis of the gases remaining at the end of a run showed that carbon monoxide, carbon dioxide, and nitrogen were present in addition to the initial gases.

The organic compounds were separated into acidic, basic, and ampholytic fractions by various ion-exchange resins. The different acids were separated by chromatography on silica (Bulen *et al.*, 1952) and the amino acids by chromatography on Dowex 50 (Stein and Moore, 1949). The compounds were identified by R_f values on ion-exchange resins, silica, and paper. Some of the compounds were characterized further by preparation of derivatives and the comparison of their melting points and mixed melting points with authentic samples of the derivative.

The optical rotation of a sample of alanine was 0.000 $\pm$ 0.003°. If this sample had been either pure enantiomorph, the rotation would have been 0.12°. The yields of compounds from the various runs are shown in TABLE 1.

Absence of Purines and Pyrimidines

The mixture of compounds from a run similar to Run 1 was evaporated to dryness, and the whole sample was chromatographed on a column of Dowex 50 (H^+) (Wall, 1953). The eluent showed no 260 mμ absorption maximum where the naturally occurring purines and pyrimidines would be eluted, and paper chromatography of the evaporated fractions showed no spots under ultraviolet radiation. It is concluded that the presence of any purine was less than 0.2×10^{-5} mols and of any pyrimidine was less than 0.1×10^{-5} mols.

The Effect of Adding Ferrous Ammonium Sulfate

Since iron is one of the more abundant elements on the earth, and would be present as both the native metal and as ferrous compounds, an experiment was performed to compare the organic compounds synthesized by a spark discharge in the system with and without added ferrous ammonium sulfate. About 16 per cent of the iron had been oxidized to ferric by the end of the run. The organic compounds were the same as in Run 1, and the quantitative values were only slightly different.

TABLE 1

YIELDS OF COMPOUNDS (MOLS × 10^5)

	Spark Run 1	Silent Run 3	N_2 run Run 6
Glycine	63 (2.1)*	80 (0.46)*	14.2 (0.48)*
Alanine	34	9	1.0
Sarcosine	5	86	1.5
β-Alanine	15	4	7.0
α-Aminobutyric acid	5	1	—
N-Methylalanine	1	12.5	—
Aspartic acid	0.4	0.2	0.3
Glutamic acid	0.6	0.5	0.5
Iminodiacetic acid	5.5	0.3	3.9
Iminoacetic-propionic acid	1.5	—	—
Formic acid	233	149	135
Acetic acid	15.2	135	41
Propionic acid	12.6	19	22
Glycolic acid	56	28	32
Lactic acid	31	4.3	1.5
α-Hydroxybutyric acid	5	1	—
Succinic acid	3.8	—	2
Urea	2	—	2
Methylurea	1.5	—	0.5
Sum of yields of compounds listed	15%	3%	8%

* Percentage yield of glycine, based on carbon placed in the apparatus as methane.

SILENT DISCHARGE: RUN 3

An experiment was performed using a silent electrical discharge (ozonizer) instead of a spark. As seen in TABLE 1, the yields are about one fourth those obtained with the use of the spark, but the products are similar.

SPARKING A MIXTURE OF METHANE, NITROGEN, WATER, AND HYDROGEN: RUN 6

The equilibrium constant for the reaction $N_2 + 3H_2 = 2NH_3$ is 7×10^5 atm.$^{-2}$, at 25° C., which predicts that the nitrogen would remain as ammonia instead of N_2 until the partial pressure of hydrogen fell below 10^{-2} atm. by escape into outer space. However, the disruptive effect of ultraviolet light and electrical discharges might result in a steady-state concentration of ammonia less than the equilibrium value. To see which organic compound would be formed under these conditions, a mixture of methane, nitrogen, hydrogen, and water was subjected to the spark. The same products are formed as in Run 1, but the yields are somewhat less.

THE MECHANISM OF SYNTHESIS

There is the question of whether the compounds observed in this system were synthesized by microorganisms. To check this point, blank runs were made with the same gases but with no spark. The production of amino acids was less than 10 μg. There is the possibility that the microorganisms might synthesize amino acids from some of the products of the discharge. To check

this point the apparatus was filled with water and with the reduced gases, and then sealed, autoclaved for 18 hours at 130° C., and sparked for one week. The yield of organic compounds was the same as that of the runs performed without autoclaving. In addition, the temperature of the apparatus was maintained at 80 to 100° C. during the run; the alanine was racemic; and the organic compounds do not represent the distribution one would expect if they had been produced by living organisms. For these reasons it is stated with confidence that the organic compounds in the system were synthesized without the aid of microorganisms.

The next problem in attempting to understand the chemistry of the system is to determine which compounds are formed in the electrical discharge, and which reactions occur in the solution phase of the system. The following alternative hypotheses will be made for the synthesis of the products.

(1) Hydrogen cyanide, aldehydes, acrylonitrile, aliphatic nitriles, amines, and part of the polymers are synthesized in the electrical discharge, and the amino, hydroxy, and aliphatic acids are formed by hydrolysis of the respective nitriles in the solution.

(2) All of the products identified were synthesized in the gas phase from radicals and ions formed in the electrical discharge.

In order to determine a few of the direct products of the electrical discharge, samples were withdrawn from the U-tube during the course of a run. Hydrogen cyanide was detected qualitatively by the Prussian blue test and estimated by titration with $AgNO_3$. Formaldehyde was detected qualitatively by chromatropic acid and acetaldehyde by *p*-hydroxydiphenyl (Neidig and Hess, 1952). The total aldehydes (and ketones) were estimated with 2,4-dinitrophenylhydrazine (Lappin and Clark, 1951).

FIGURE 2 shows the concentrations of ammonia, hydrogen cyanide, and aldehydes in the U-tube and amino acids in the 500 ml. flask during the sparking of a mixture of methane, ammonia, water, and hydrogen. It is seen that the concentration of ammonia decreased steadily during the run, mostly because of the decomposition of the ammonia to hydrogen and nitrogen in the discharge. The hydrogen cyanide concentration rose to 4×10^{-2} M and, after 120 hours, apparently little more was synthesized in the spark. Thereafter, the hydrogen cyanide present was hydrolyzed to formic acid or decomposed in the spark. The aldehyde concentration rose to about 10^{-3} M and declined after 120 hours. The concentration of amino acids rose during the run and leveled off after about 140 hours.

Several repetitions of this experiment gave concentrations of these compounds of the same order of magnitude, but the values were not reproducible in detail. Probably the most important variable that could not be controlled was the operation of the spark.

Hydrolysis of Aminonitriles and Hydroxynitriles: Run 4

Hydrogen cyanide, aldehydes, and ammonia are known to react to give aminonitriles and hydroxynitriles. It is uncertain whether the conditions of this experiment will hydrolyze these nitriles to the corresponding acid. A solution of 63 mmols NH_3 (corresponding to 25 cm. Hg pressure), 20 mmols

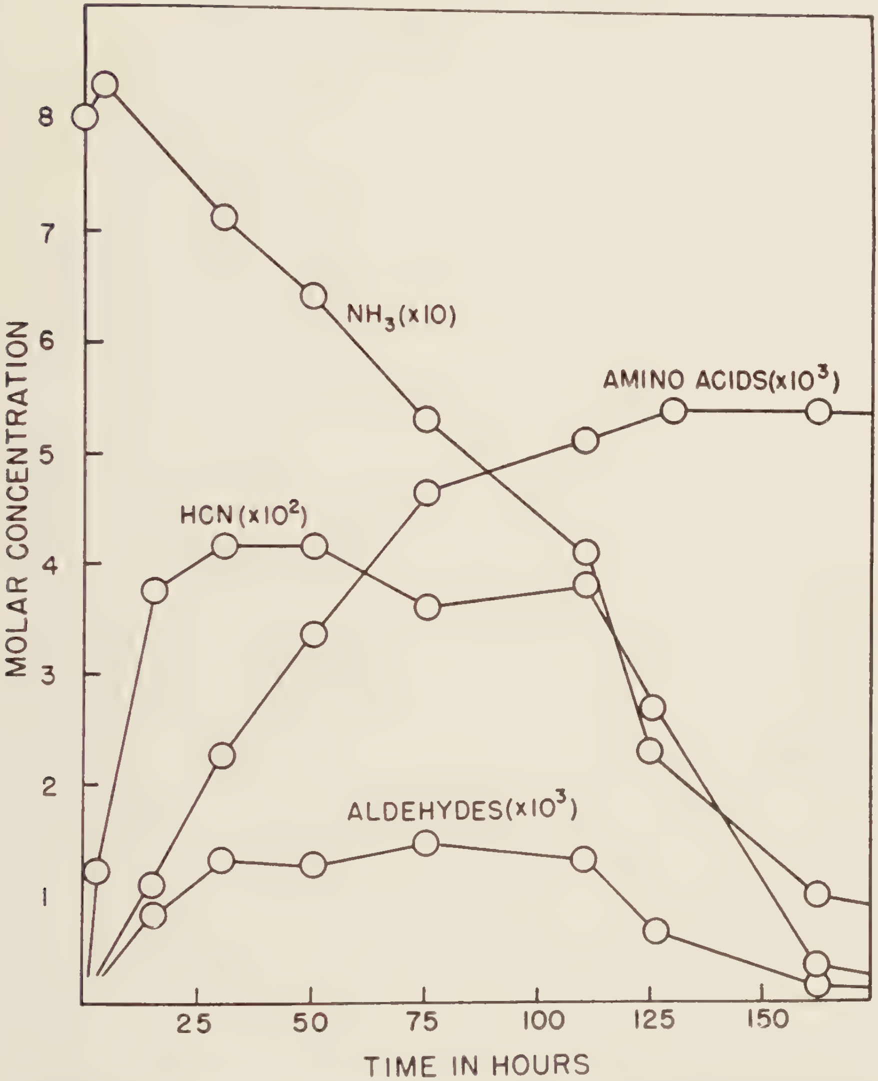

FIGURE 2. The concentrations of ammonia, hydrogen cyanide, and aldehydes in the U-tube, and of amino acids in the 500 ml. flask while sparking a mixture of methane, ammonia, water, and hydrogen in the apparatus shown in FIGURE 1.

hydrogen cyanide, 6.1 mmols formaldehyde, 3.64 mmols acetaldehyde, and 1.16 mmols propionaldehyde (in 325 ml. H_2O) was boiled in the apparatus for one week. The glycine, glycolic acid, iminodiacetic acid and iminoacetic-propionic acid accounted for 52 per cent of the formaldehyde; alanine, lactic acid, and iminoacetic-propionic acid accounted for 58 per cent of the acetaldehyde; α-amino-n-butyric acid and α-hydroxybutyric acid accounted for 36 per cent of the propionaldehyde. This experiment shows that aminonitriles and

hydroxynitriles can be hydrolyzed in this system and, further, that the acids are formed in good yield from the aldehyde.

By titrating samples withdrawn from the U-tube for hydrogen cyanide during the course of the run, the rate constant for hydrolysis of hydrogen cyanide to formic acid is estimated to be 0.1 hr.$^{-1}$. Similarly, by determining the amino acid concentration in the 500 ml. flask at various times, the rate constant for hydrolysis of the aminonitriles is estimated to be 0.2 hr.$^{-1}$.

From these rate data and from the concentrations in FIGURE 2 the yield of formic acid from hydrolysis of hydrogen cyanide is calculated to be 3.6 mmols, and the yield of amino acids from hydrolysis of the aminonitriles is calculated to be 1.4 mmols. These values agree within the experimental error with the observed yields of 2.4 mmols of formic acid and 1.2 mmols of amino acids. Thus, in the case of the spark discharge, the rates of hydrolysis under the conditions of the experiment are sufficient to account for the total yields of formic acid and amino acids observed.

The Ratios of Products

Further evidence that the amino and hydroxy acids are synthesized through the corresponding nitrile can be obtained by consideration of the ratios of products predicted by this mechanism.

It can be shown (Miller, 1955) that if the reaction of the aldehyde, hydrogen cyanide, and ammonia to form the aminonitriles and hydroxynitriles is a rapid and reversible equilibrium, and that the hydrolysis is a first-order irreversible reaction, then the ratio of hydroxy acid to amino acid at the end of the run will be

$$R_1 = h_iH_i/k_iK_i(\mathrm{NH_3}) \qquad (1)$$

where H_i and K_i are the equilibrium constants for the formation of the hydroxy- and aminonitrile from aldehyde$_i$, and h_i and k_i are the respective rates of hydrolysis. Similarly, we have

$$R_2 = N\text{-methylamino acid/amino acid} \qquad (2)$$

$$= m_iM_i(\mathrm{CH_3NH_2})/k_iK_i(\mathrm{NH_3})$$

where M_i and m_i are the equilibrium constant and rate constant of hydrolysis of the methylaminonitrile.

β-Alanine cannot arise from a Strecker synthesis as with the α-amino acids. A reasonable mechanism would be from a Michael addition of ammonia to acrylonitrile, acrylamide, or acrylic acid. One would expect that hydrogen cyanide and methylamine would also add to give, after hydrolysis, succinic acid and N-methyl-β-alanine. This last compound was not detected during the analysis, since it does not react with ninhydrin. The rate of formation of the nitrile of β-alanine and succinonitrile would be $k_{\mathrm{NH_3}}(\mathrm{NH_3})(\mathrm{CH_2{=}CHCN})$ and $k_{\mathrm{HCN}}(\mathrm{CN^-})(\mathrm{CH_2{=}CHCN})$, respectively, where the k's are the rate constants for addition. Assuming that the addition is irreversible, and that the

TABLE 2
RATIOS OF PRODUCTS

	Spark Run 1	Silent Run 3	N_2 run Run 6	Aldehydes HCN, NH_3 Run 4
Glycolic/Glycine	0.89	0.35	2.3	0.73
Lactic/Alanine	0.91	0.48	1.5	0.33
Hydroxybutyric/Aminobutyric	1.0	1.0	—	0.55
Sarcosine/Glycine	0.08	1.07	0.11	—
Methylalanine/Alanine	0.03	1.4	—	—
Succinic/β-Alanine	0.25	—	0.29	—

nitriles are hydrolyzed by the end of the run, then

$$(\text{succinic acid})/(\beta\text{-alanine}) = k_{HCN}(CN^-)/k_{NH_3}(NH_3) \qquad (3)$$

This treatment is easily generalized to include additions to acrylonitrile and acrylic acid. The ratios of products are given in TABLE 2.

If the ratio hH/kK does not depend on the aldehyde, then EQUATION 1 predicts that the ratio of the hydroxy acid to the amino acid should be the same for the different aldehydes in a given run. The agreement is good for the spark discharge and silent discharge except for the hydroxybutyric/aminobutyric in the silent discharge. In Run 4 the hydrolysis of the aminonitriles and hydroxynitriles was necessarily the mechanism for synthesis of the respective acids. There is less agreement of the ratios than with the electric discharges, but the agreement is within the errors of the experiment.

Similarly, the ratios of methylamino acid/amino acid are nearly the same for Runs 1 and 3. The succinic acid/β-alanine ratio is the same in Runs 1 and 6.

The ratios of various products are in qualitative agreement in all cases and in quantitative agreement (within the experimental error) in most of the cases. The similarity of products in Runs 1 and 4 is striking (except for the expected absence of β-alanine and succinic acid, since no acrylonitrile was added), which suggests that the products were formed by the same mechanism.

Since the production of aldehydes and hydrogen cyanide is sufficient to account for the observed yield of amino acid, there can be little doubt that most of the amino and hydroxy acids were formed from the nitriles in Run 1. However, these experiments do not exclude the possibility that a small per-

centage of the amino acids were formed directly in the spark, entirely by radical reactions.*

The synthesis of the products expected from acrylonitrile and the agreement of the ratios of these products in the different runs provides strong indirect evidence for the synthesis of β-alanine and succinic acid by β-addition and, in turn, that acrylonitrile or its derivatives were synthesized in the electric discharges.

If cyanate were formed in the electrical discharge, then both urea and methylurea could be expected by reaction with ammonia and methylamine (Wöhler synthesis). The direct synthesis of the simple ureas in the electrical discharge is also quite reasonable.

Discussion

Assuming that the earth initially had a reducing atmosphere, do the experimental results obtained in this very simple system show that amino acids and other organic compounds would be present in the oceans? The experiments on the mechanism of the electric discharge synthesis of amino acids indicate that a special set of conditions or type of electrical discharge is not required to obtain amino acids. Any process or combination of processes that yielded both aldehydes and hydrogen cyanide would have contributed to the amount of α-amino acids in the hydrosphere of the primitive earth. Therefore, electrical discharges are not critical for the synthesis of amino acids, and the similar results could be expected from ultraviolet light.

The ultraviolet light emitted by the sun as black-body radiation amounts to 85 cal. cm.$^{-2}$ yr.$^{-1}$ for wave lengths less than 2000 Å. and 1.6 cal. cm.$^{-2}$ yr.$^{-1}$ for wave lengths less than 1500 Å. (Urey, 1952b). Superimposed on the black-body radiation is a strong Lyman α line at 1216 Å. of 1.9 cal. cm.$^{-2}$ yr.$^{-1}$ (Rense, 1953). This line is absorbed by CH_4, H_2O, NH_3 and CO.

Hydrogen atoms from the photolysis of CH_4, NH_3 and H_2O would react with CO to give formaldehyde (Caress and Rideal, 1928; Taylor, 1926). Carbon monoxide activated by wave lengths of less than 1545 Å. reacts with H_2 to give formaldehyde and glyoxal (Groth, 1937). Hydroxyl radicals would react with hydrocarbons to give aldehydes (Milas, Stahl, and Dayton, 1949). If any O atoms should be formed by photolysis of water or CO, they would react rapidly with H_2 to give H_2O and with hydrocarbons to give aldehydes.†

Active nitrogen, probably N atoms in the ^4S state (Jackson and Schiff, 1955) reacts with methane and other hydrocarbons to give hydrogen cyanide in good yield (Winkler and Schiff, 1953). Photodissociation of N_2 (< 1100 Å.) or NH radicals gives N atoms. NH and NH_2 radicals from the photolysis of

* The hydrogen cyanide concentration in the silent discharge case is too low to account for the yield of amino acids unless the hydrolysis of the nitriles is more rapid than in Run 1. Hydrogen peroxide catalysis is a possibility. The ratios of products in the various runs is strong evidence that the products were formed by the same mechanism.

† If aldehydes were synthesized from the Lyman α radiation with a quantum yield of 1.0, the yield for the earth would be 2×10^{13} mols yr.$^{-1}$. If the aldehydes were dissolved in the present oceans this would give a solution of 3×10^{-8} M. Of course, the efficiency of the Lyman α radiation would not have been 100 per cent, but the oceans probably would have been smaller in volume, and the electrical discharges and high temperature reactions would contribute to the production of aldehyde.

ammonia might react with hydrocarbons to give hydrogen cyanide, but this has not yet been demonstrated.

The reactions outlined above show that aldehydes and hydrogen cyanide would be produced photochemically, and there probably are other photochemical reactions that would also give these compounds.*

Infrared radiation by the polyatomic molecules of the reducing atmosphere probably would result in a cool atmosphere and ocean rather than the boiling temperatures used in these experiments or the molten earth proposed by some workers. However, if there were any local areas of high temperature, hydrogen cyanide would be formed (Migrdichian, 1947), and aldehydes might be synthesized from hydrocarbons and carbon monoxide by reactions analogous to the Fischer-Tropsch or hydroformalation reactions (Storch, Golumbic, and Anderson, 1951).

If conditions on the earth were cool, then the hydrolysis of the nitriles would still take place, but more slowly than in these experiments. The Strecker synthesis of amino acids will work at much lower concentrations of aldehyde and hydrogen cyanide than obtained in these experiments. At very low concentrations, however, the Strecker synthesis will not operate. The rate of synthesis of amino acid is given by

$$-d(\mathrm{HCN})/dt = kK(\mathrm{NH_3})\,(\mathrm{RCHO})\,(\mathrm{HCN}) \tag{4}$$

The $kK(\mathrm{RCHO})$ means the sum of this term over the different aldehydes. The hydrolysis of hydrogen cyanide to formic acid is a competing reaction with the rate

$$-d(\mathrm{HCN})/dt = r(\mathrm{HCN}) \tag{5}$$

where r is the rate constant for the hydrolysis of hydrogen cyanide. Thus, if the concentrations of aldehydes are so low that $kK(\mathrm{NH_3})(\mathrm{RCHO})/r \ll 1$, then cyanide will not be available for the Strecker synthesis because of hydrolysis to formic acid. It is necessary to know the values of K, k, H, h, and r, their pH, and their temperature dependence for a quantitative treatment of this problem.

From a qualitative standpoint it can be seen that the Strecker synthesis will operate in very dilute solutions. The H for acetaldehyde at 25° C. is 1.4×10^4 (Yates, 1952) and the K is probably greater. The experiments reported here indicate that h, k, and r are of the same order of magnitude. Thus, if the hydrolysis of the nitriles in the hydrosphere is by the same mechanism as in these experiments (probably OH^- attack on the carbon of the nitrile) then (RCHO) can be as low as 10^{-4}–10^{-5} M, and the Strecker synthesis will still operate. If the value of k relative to r (and h) is increased by catalytic hydrolysis (for example, SH^-, $HPO_4^{=}$), then the concentration of aldehydes could be much lower.

* Some preliminary experiments performed at Brookhaven National Laboratory, Upton, N. Y., showed that the 1850 A. mercury line can synthesize amino acids from CH_4, NH_3 and H_2O. Only NH_3 and H_2O absorb this line, but apparently the radical reactions formed the active carbon intermediates. Formaldehyde was detected. The yield of amino acids was very low.

The ratio of hydroxy acid to amino acid is given by EQUATION 1. If the concentration of ammonia is very low and (RCHO) and (HCN) are sufficiently high, then hydroxy acid rather than amino acid or formic acid will be synthesized.

There are competing reactions that the aldehyde can undergo instead of a Strecker synthesis. The aldehydes can be reduced or oxidized, the latter being important if any oxygen were present. The most important competing reaction would be aldol condensations. These condensations would give biologically important products such as trioses, tetroses, pentoses, and hexoses. The rate of these condensations relative to the Strecker synthesis would not depend markedly on the concentrations of aldehydes, since the aldol condensations would be second-order reactions. Therefore, the competing reactions of the aldehydes would not predominate at low concentrations.

In the above discussion the composition of the primitive atmosphere of our planet has been assumed to be reducing. The general geochemical argument for the reducing atmosphere given by Oparin and by Urey is that the ratio of hydrogen to oxygen in the universe is about 1000:1, the earth being rather anomalous in this respect. No one has demonstrated any mechanism that, before the planets were formed, would produce oxygen in the region of the earth, but not in the region beyond Mars. The formation of oxidizing conditions on Mercury, Venus, Earth, and Mars after their formation is explained by the escape of hydrogen from these planets. Their atmospheres are sufficiently hot and their gravitational fields sufficiently weak to allow hydrogen to escape into outer space from the atmosphere. The escape of the strong reducing agent, H_2, results in an oxidizing atmosphere. In the region beyond Mars the planets have low temperatures and high gravitational fields. These conditions prohibit the escape of hydrogen from their atmospheres, as a result of which they are still reducing.

A second argument for the existence of a reducing atmosphere on the primitive earth is based on the assumption that, for life to arise, there must be present, first, a large number of organic compounds similar to those that would make up the first organism. Therefore, if it can be demonstrated that the organic compounds that make up living systems *cannot* be synthesized under oxidizing conditions, and if it can be shown that these organic compounds *can* be synthesized under reducing conditions, then one conclusion would be that the earth had a reducing atmosphere in its early stages and that life arose from the sea of organic compounds that was formed while the earth had such an atmosphere.

From a review of the literature on electrical discharges (Glockler and Lind, 1939) and ultraviolet light (Noyes and Leighton, 1941), from the results of the experiments described in this paper, and from the first part of this discussion, one can see that organic compounds can be synthesized easily under reducing conditions.

There have been many attempts to synthesize organic compounds under oxidizing conditions, usually from carbon dioxide and water, and these attempts almost always have failed. A review of such attempts, in which ul-

traviolet light was used (Rabinowitch, 1945), shows that success was claimed by some workers, but when their experiments were repeated in other laboratories or when contaminating reducing agents were removed, no organic compounds were synthesized. The action of electrical discharges on carbon dioxide and water also has resulted in failure (Wilde, Zwolinski, and Parlin, 1953). Of course, if a strong reducing agent such as Na or Mg is used, organic compounds can be formed, but these reducing agents would not be present on the earth with either a reducing or an oxidizing atmosphere. High-energy radiations on ammonium carbonate solutions might give organic compounds, but the presence of ammonia would imply reducing conditions.

There has been one successful synthesis of organic compounds from carbon dioxide and water. This was done with 40 million electron-volt helium ions from a 60-inch cyclotron (Garrison *et al.*, 1951; Garrison and Rollefson, 1952). Formic acid was obtained in small yield and, if ferrous iron was added to the solution (as a reducing agent), then a small yield of formaldehyde was obtained in addition to formic acid. In view of the absence of a strong source of high-energy particles, the small yields, and the very simple organic compounds synthesized, it would seem that instead of showing that organic compounds can be synthesized, this experiment is an excellent demonstration that organic compounds cannot be synthesized effectively under oxidizing conditions.

If any organic compounds should be synthesized under oxidizing conditions, however difficult this may be, then the question of their stability would arise. In the presence of molecular oxygen the organic compounds would be oxidized rather rapidly, especially in the presence of light (Palit and Dhar, 1930). An important reaction of the oxygen would be the oxidative deamination of the amino acids. This reaction is catalyzed by blood charcoal and, probably, by many iron compounds (Warburg, 1949). The oxidative deamination is a significant reaction even in the absence of catalysts (Abelson, 1956b). Oxygen also would attack aromatic compounds such as the purines and pyrimidines, especially in the presence of light. These arguments make a strong case that free oxygen must have been absent when the organic compounds were formed as well as during the development of heterotrophic organisms. Shortly after the appearance of oxygen on the earth the autotrophic organisms must have developed, for otherwise the nutrients would have been exhausted rapidly.

If the Strecker synthesis was the principal synthesis of amino acids on the primitive earth, then ammonia must have been present in the ocean, even though N_2 could have been the principal nitrogen species in the atmosphere.* This implies that the earth must have been rather reducing, with a pressure of N_2 of at least 10^{-3} atmospheres, unless one is to assume that the amino acids were formed in limited areas that contained reducing conditions.

* Ammonia is quite soluble in water. The vapor pressure in atmospheres is given by $p_{NH_3} = \alpha[(NH_4OH) + (NH_4^+)]$, where the concentrations of NH_4OH and NH_4^+ are in mols/liter. For 25° C., α is 9.3×10^{-5} at $pH = 7$, 8.8×10^{-4} at $pH = 8$, and 6.1×10^{-3} at $pH = 9$. Thus, unless the temperature of the ocean was rather high (70–100° C.), most of the ammonia would be in the ocean. The cyanide could be formed from the N_2 in the atmosphere (as happened in Run 6).

This argument would not be valid if there are other reasonable syntheses of amino acids. One possibility would be the reductive amination of any α-keto acids present in the ocean, although decarboxylation of the keto acid would be a competing reaction. Another reaction would be synthesis of amino acids from α-keto aldehydes and ammonia catalyzed by mercaptans (Wieland, Franz, and Pfleiderer, 1955; Wieland and Jaenicke, 1955). A possible source of the α-keto aldehydes would be from the oxidation of polyhydroxyl compounds obtained from aldehyde condensations. These two syntheses require ammonia, however. It is very difficult to see how an amino group can be synthesized directly from N_2 by any reasonable process except under reducing conditions. Reasonable syntheses of amino acids involving hydroxylamine, nitrites, or nitrates would require strong reducing agents to convert the nitrogen to an amino group. A direct synthesis of the amino acids in an electrical discharge, if possible, probably would require reducing conditions.

On the basis of primarily geochemical arguments, Rubey (1955) has contended that the primitive earth had an atmosphere of carbon dioxide, nitrogen, carbon monoxide, and water. This atmosphere would come mainly from the interior of the earth rather than be composed of the residual gases of the cosmic dust cloud. Abelson (1956a) has examined the action of a spark discharge on this mixture of gases and found that good yields of amino acids could be obtained as long as some hydrogen was present.* Conversely, when no hydrogen was present, no amino acids were obtained. The production of amino acids was more rapid if CO_2, H_2O, and NH_3 (instead of N_2) were used. The mechanism of the reaction was not investigated, but it may well be a Strecker synthesis as in the case of methane, ammonia, and water.

Because of the presence of hydrogen in the gas mixtures used by Abelson, the mixtures were reducing, although not nearly to the same degree as the mixture of methane, ammonia, water, and hydrogen. Therefore, the argument that reducing conditions are necessary to synthesize organic compounds is not altered, but whether the atmosphere was strongly reducing or only weakly so cannot be decided on the basis of ability to synthesize organic compounds.

As hydrogen escapes into outer space from a strongly reducing atmosphere, such an atmosphere would become less reducing and, finally, become oxidizing. Thus, the atmosphere proposed by Urey would be converted in the course of time to that proposed by Rubey. The principal question involves the relative lengths of time that the earth had these respective atmospheres. This question is not critical to the problem of spontaneous generation, since organic compounds can be synthesized in both proposed atmospheres (provided that the one suggested by Rubey contains some hydrogen), and since the organic compounds would be similar in both cases.

* The equilibrium constant for the reaction

$$CO_2 + 4H_2 = CH_4 + 2H_2O$$

is 7×10^{21} at 25° C., so that the mixture of gases used by Abelson is thermodynamically unstable. Whether this equilibrium would be attained on the earth is not predicted by thermodynamics, but carbon dioxide in the presence of hydrogen can be reduced to carbon monoxide and methane in electrical discharges and probably by ultraviolet light.

Summary

(1) Experiments are described showing that electrical discharges in a mixture of methane, ammonia, hydrogen, and water will produce amino, hydroxy, and aliphatic acids. This mixture of gases has been proposed as the composition of the earth's atmosphere in the early stages of formation.

(2) The same compounds (but in different yields) are formed with both spark and silent discharges; when methane, nitrogen, water, and hydrogen are sparked; and when ferrous ammonium sulfate is added to the system.

(3) Hydrogen cyanide and aldehydes are direct products of the electrical discharge. These compounds react to give amino- and hydroxynitriles, which are then hydrolyzed to the corresponding acid (Strecker synthesis) in the aqueous phase of the system.

(4) Arguments are presented to show that the same types of compounds would be synthesized if the earth had a reducing atmosphere. Both ultraviolet light and electrical discharges would produce aldehydes and hydrogen cyanide, and therefore would contribute to the amount of amino acids in the ocean.

(5) It is probable that the Strecker synthesis will operate at the low concentrations of aldehydes and hydrogen cyanide that might be expected in the oceans of the primitive earth.

(6) It is pointed out that organic compounds would not be synthesized on the earth if oxidizing conditions were present. Therefore, if one assumes that amino acids (and other organic compounds) must be present for life to arise, then the atmosphere of the earth must have been reducing. In particular, ammonia must have been present in the oceans for the synthesis of amino acids. This implies that the partial pressure of hydrogen was at least 10^{-3} atmospheres.

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Discussion of the Paper

PHILIP H. ABELSON (*Geophysical Laboratory, Carnegie Institution of Washington, D. C.*): Stanley Miller's contributions in his study of the synthesis of amino acids from mixtures of methane, ammonia, and water have been important and significant. However, there has been a tendency for others to overinterpret his findings. I have heard thoughtful scientists conclude that, because amino acids could be synthesized from such a mixture, the early atmosphere of the earth must have been composed of methane, ammonia, and water.

To explore this suggestion, the effects of electrical discharges on twenty different gaseous mixtures have been studied with equipment similar to that employed by Miller. Various proportions of methane, ammonia, and water; carbon dioxide, nitrogen, and water; carbon dioxide, nitrogen, hydrogen, and water; methane, nitrogen, and water; carbon dioxide, ammonia, hydrogen, and water; and carbon monoxide, nitrogen, hydrogen, and water were tested.

Amino acids, including glycine, sarcosine, alanine, and β-alanine, were synthesized with these compositions, with two notable exceptions. When proportions of methane and ammonia were high with respect to water vapor, and when the mixture of carbon dioxide, nitrogen, and water was employed, amino acids were not produced. In general, the atomic proportions of the components in the other mixtures were chosen to be proportional to those found in glycine; namely, 2-C, 1-N, 2-O, and 5-H. However, it was found that wide variations in carbon-nitrogen ratio could be tolerated.

A tremendous volume of geologic evidence[1] exists concerning the past history of the earth. W. W. Rubey has summarized that part of it that bears on the evolution of the sea and the atmosphere. He concludes that the ocean and air were formed as products of the degassing of the interior of the earth. Evidence for volcanic activity is found in the earliest rocks. Gases associated with present-day eruptions include water, carbon dioxide, nitrogen, carbon monoxide, hydrogen, and sulfur. Condensation and absorption of such a mixture lead to an atmosphere composed of carbon monoxide, nitrogen, hydrogen, and small amounts of water and carbon dioxide.

Further adjustments of composition would occur under the influence of ultraviolet light and in the presence of a reducing crust. Energy associated with electrical discharges is very small in comparison with the energy present in visible and ultraviolet radiation. Later experiments may well show that the major synthetic mechanisms probably involved ultraviolet light.

These investigations will have lasting significance concerning the origin of life insofar as they are related to what, on geologic grounds, was the real primitive world. Ideally, the laboratory should predict or explain compounds that one day will be found in early sedimentary rocks.

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SOME ASPECTS OF PALEOBIOCHEMISTRY

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Organic chemicals of biological origin are being found in an increasing number of fossils, sediments, and sedimentary rocks. The existence of these paleobiochemicals has special significance in many areas of science. It raises real possibilities of achieving better knowledge of the comparative biochemistry of creatures long extinct, including some of the earliest forms of life. The occurrence of these ancient substances permits study of organic chemicals under circumstances in which the rate of reaction is known to be less than one part in 10^{17} per second. Knowledge of the stability of these compounds is important to the question of the origin of life, since most speculations assume the accumulation of a pool of organic substance during a long pre-life stage.

Environments of Preservation

The usual fate of organic molecules is participation in the carbon cycle and metabolic oxidation to carbon dioxide. However, special circumstances, including low temperature, absence of free oxygen or water, and protection from bacterial attack, favor preservation.

The well-known preservation of mammoth flesh in Arctic ice illustrates the favorable effect of low temperature. Such an environment stops bacterial action and slows hydrolysis, whether induced chemically or enzymatically.

Boyd and Boyd[1] have been able to determine the blood types of Egyptian mummies. The excellent preservative that makes this possible is undoubtedly the dry environment, which inhibited both hydrolysis and bacterial action.

Thieme, Otten, and Sutton[2] have examined bones of early American Indians, 10,000 years old or more, from Midland, Texas, and have been able to obtain definitive serologic reactions of blood types. In this case the crucial factor seems to be the protection from bacterial attack offered by some parts of bone. My own studies of fossil shells also show well-preserved late Pleistocene proteins. Again, the important factor is freedom from bacterial attack. Perhaps a variety of circumstances favors the preservation of exceptionally large quantities of long peptide chains in bones taken from the La Brea tar pits in Los Angeles, Calif. These have an amino acid content of 10 per cent. Here the bones have been immersed in asphalt, and the environment is anaerobic and relatively free of water.

There is a limitation on the stability of most proteins that probably prevents their existing for even a million years at room temperature; namely, the breakdown of some of the amino acids such as serine. Nevertheless, even within this limitation, the existence of intact or only slightly broken ancient proteins opens up many opportunities for research. Most important of these are questions that concern the recent evolution of man.

My own investigations[3-6] have been concerned principally with the amino acid constituents of fossils. Since these studies have led to findings that help illuminate some general considerations, they will be discussed in detail.

Table 1

Amino Acid Content of Various Recent Tropical Calcium Carbonate Shells

Specimen*	Description	Mineral composition	Amino acid content†
Porites lichen	Reef-building coral	Aragonite, 10 to 20 per cent calcite	6.7
Porolithon onkodes	Coralline alga	Calcite with $MgCO_3$ in solid solution	17.6
Chama lazarus	Reef-dwelling clam	Aragonite	16.4
Codakia punctata	Ribbed clam	Aragonite	8.4
Atactodea glabrata	Intertidal clam	Aragonite	42.7
Cymatium muricinum	Thick-shelled snail	Aragonite	20
Tridacna maxima	Man-eating clam	Aragonite	26.6
Cypraea mauritiana	Tropical snail	Aragonite	6.2

* The materials were furnished by Preston Cloud of the United States Geological Survey. The *Porites lichen* and *Porolithon onkodes* were collected at Saipan. The other specimens were collected at Onotoa Atoll in the Gilbert Islands.

† In micromols per gram (μM/gm.). One μM/gm. is approximately equal to 1 part per 10,000.

Fossils were chosen as test objects for two principal reasons: first, the matrix might protect organic constituents from bacterial attack; second, the validity of association of organic constituents with fossils of a given species can be checked by studying specimens obtained at other localities, thus minimizing adventitious effects.

The high organic chemical content of bones is well known. Most of this, however, is usually lost. Shells are far more available, and their initial organic content, though low, is often subject to smaller losses. It was interesting, therefore, to find that recent calcareous shells all contain protein. In TABLE 1 are shown results of measurements on a group of calcareous materials of biological origin. Tests of 100 different recent species, including foraminifera, have all revealed a measurable content of amino acid.

In some cases the shells possessed an outer covering; this was first removed. It was established by means of experiments in which the shell was dissolved in 10 per cent steps that the protein is distributed throughout the shell. This is illustrated in TABLE 2. A meshwork of protein often resulted when shells were dissolved in trichloracetic acid.

The Effect of Temperature on Preservation

Although the age of the fossil is an obvious variable in determining whether organic constituents have been preserved, it is surpassed in importance by burial history and the consequent thermal history. Some fossils are found today in formations that probably have been buried more than 20,000 feet and have been exposed to temperatures as high as 200° C. for long periods of time. Such fossils are almost certain to be recrystallized, and they do not contain amino acids.

To examine the effect of temperature on preservation, I found it particularly enlightening to conduct some accelerated aging tests on amino acids, choosing

TABLE 2
SERIAL SOLUTION OF A RECENT CLAMSHELL*

Fraction (%)	Amino acid content (μM/gm.)	Protein (%)
10–20	11.7	1.24
20–30	12.3	1.31
30–40	17.8	1.90
40–50	20.3	2.17
50–60	22.0	2.35
60–70	17.8	1.90
70–80	15.0	1.61
80–90	10.2	1.09
Average........................	15.9	1.70

* The specimen used was *Mercenaria mercenaria*, an edible clam grown at Cape May, N. J

aqueous solutions of alanine for extensive study.[4] Experiments revealed that the reaction involved is

$$CH_3-\underset{\displaystyle H}{\overset{\displaystyle NH_2}{C}}-COOH \rightarrow CH_3-\overset{\displaystyle NH_2}{CH_2} + CO_2 \qquad (1)$$

This degradation is a first-order reaction, and the rate is proportional to the concentration. A series of tests was conducted at various temperatures to determine the time θ required for initial concentrations to diminish to $1/e$ or 37 per cent. Results of such tests are shown in FIGURE 1. The upper four points on the line were determined by Dwight Conway in experiments performed at the University of Chicago, Chicago, Ill., under W. F. Libby. I am indebted to these two investigators for making these data available to me in advance of publication. Conway has used a sensitive carbon^{14} method which should prove extremely useful in following very slow reactions. It may be seen that a plot of the log of time θ against the reciprocal of absolute temperature gives a straight line. Extrapolation suggests that, near room temperatures, solutions of alanine might persist for a billion years.

On the other hand, most of the alanine would disappear in about 1000 years at 120° C. or in a few hours at 250° C. It would, of course, be desirable to study this degradation in an environment other than water. Some experiments with calcareous fossils have given results in agreement with the aqueous tests, but the effects obtained with other matrices might well be different.

The thermal stability of other amino acids was explored also. Alanine, α-aminobutyric acid, glutamic acid, glycine, isoleucine, leucine, proline, and valine were found to be most stable. Threonine, lysine, tyrosine, and phenylalanine are moderately so. Serine is easily destroyed. Aspartic acid hydrolyzes to ammonia and malic acid.

Amino Acids in Fossils

These results take on added interest against the background of the amino acid content of fossil materials. It is particularly enlightening to compare

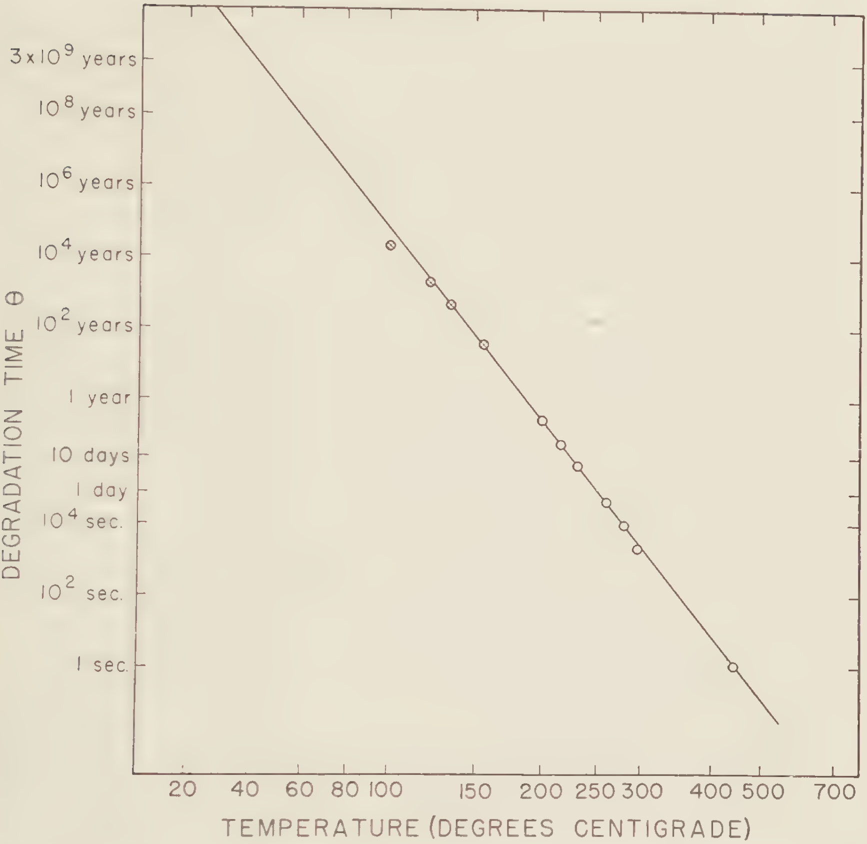

FIGURE 1. The thermal degradation of alanine as shown by accelerated tests of aging. Shown is the time required for the decomposition of 63 per cent of the original material. The upper four points were determined by the use of alanine tagged with C^{14} by Dwight Conway at the University of Chicago.

the amino acids of recent shells with those of fossil representatives of the same species. For this purpose the hard-shelled clam *Mercenaria mercenaria* is particularly useful. This creature is abundant today along the east coast of the United States. Fossil specimens have been found in many localities of various formations covering a period of more than 25,000,000 years. A continuous series of fossils of Pleistocene, Pliocene, and Miocene age may be studied, and the changes with time followed. In FIGURES 2 and 3 are displayed chromatograms of amino acids obtained from a recent shell and from another that was 25,000,000 years old. It may be noted that the principal components of the fossil are alanine, glutamic acid, glycine, isoleucine or leucine, proline, and valine.

With this background it was also interesting to examine the amino acid content of some bones and other shells. TABLE 3 lists findings obtained from a series of fossils chosen on the basis of probable moderate thermal history.

FIGURE 2. Hydrolysate of protein isolated from a recent shell of *Mercenaria mercenaria*. The abbreviations used in the photograph are: ALA, alanine; ASP, aspartic acid; GLU, glutamic acid; GLY, glycine; ILEU, isoleucine; LEU, leucine; LYS, lysine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TYR, tyrosine; VAL, valine.

This series represents only a small fraction of the fossils that have been studied. The same general pattern of amino acids was found in all cases where the depth of burial had been minimal and the fossils were not recrystallized.

It seems reasonably certain that all living species and many extinct creatures, such as the dinosaur, have used the same amino acid building blocks in performing their vital functions. It is perhaps of some interest to know that no amino acids other than those used today were found. For instance, α-aminobutyric acid, which undoubtedly is stable enough to persist, was not found in quantity. The possibility does exist, of course, that a wide canvass of fossils might point to exceptions or to the presence of other amino acids not used today.

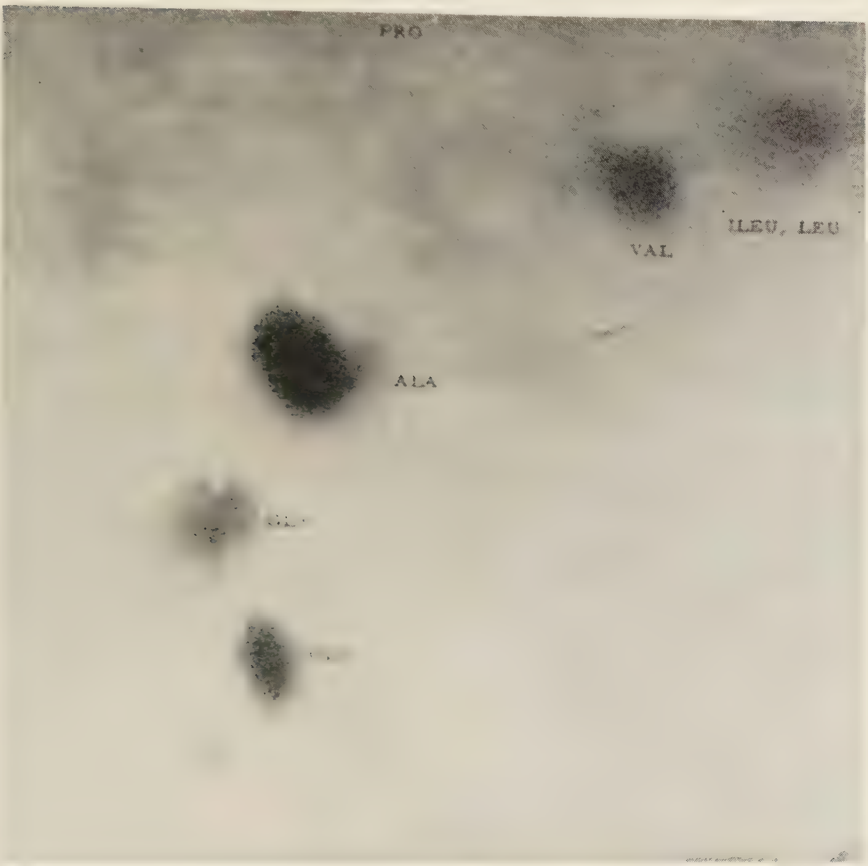

FIGURE 3. Amino acids found in Miocene (25,000,000 year old) shell of *Mercenaria mercenaria*. The abbreviations used in the photograph are: ALA, alanine; GLU, glutamic acid; GLY, glycine; ILEU, isoleucine; LEU, leucine; PRO, proline.

The Effect of Oxygen on Amino Acids

In view of the general topic of this monograph, it is perhaps desirable to mention some experiments on the effects of oxygen on solutions of amino acids. The studies are still only fragmentary, but they point clearly to a major influence of this gas on rates of degradation. In the experiment, ten micromolar solutions of the respective amino acids were loaded in glass ampoules under an atmosphere of oxygen. The tubes were sealed and then exposed to the temperature indicated. In control runs the ampoule was evacuated before closure.

In TABLE 4 it can be seen that degradation was greatly enhanced in the presence of oxygen. The relative effect was greater with alanine at lower temperatures. Extrapolation of the effects to room temperature leads to the

TABLE 3
AMINO ACID CONTENT OF VARIOUS FOSSILS

Name	Approximate age (years)	Formation	Amino acid content (μM/gm.)	Principal constituents*
Plesippus (prehistoric horse, tooth)	Late Pliocene 5×10^6	Hagerman Lake Beds, Idaho	1.5	Gly, ala, leu, val, glu
Glycymeris parilis (clam)	Miocene 25×10^6	Calvert, Chesapeake Bay, Md.	0.80	Ala, gly, glu, leu, val, pro
Ecphoratricostata (snail)	Miocene 25×10^6	Calvert, Chesapeake Bay, Md.	1.20	Ala, gly, glu, leu, val, pro
Mosasaurus (dinosaur)	Cretaceous 100×10^6	Pierre Shale, S. Dak.	1.8	Ala, gly, glu, leu, val
Anatosaurus (dinosaur)	Cretaceous 100×10^6	Lance, Lance Creek, Wyo.	2.8	Ala, gly, glu, leu, val, asp
Stegosaurus (dinosaur)	Jurassic 150×10^6	Morrison, Como Bluff, Wyo.	0.26	Ala, gly, glu
Dinichthys (fish)	Devonian 360×10^6	Ohio Black Shale	3.0	Gly, ala, glu, leu, val, asp
Plaesiomys subquadrata (brachiopod)	Ordovician 430×10^6	Waynesville, Oxford, Ohio	0.50	Ala, gly, glu, leu, val

* Abbreviations: Ala, alanine; asp, aspartic acid; glu, glutamic acid; gly, glycine; leu, leucine or isoleucine; pro, proline; val, valine.

TABLE 4
EFFECT OF OXYGEN ON AMINO ACIDS

Amino Acid	Temp. (°C.)	Oxygen	Degradation time θ (sec.)
Serine	229	yes	1900
Serine	229	no	4150
Threonine	231	yes	3000
Threonine	231	no	18,000
Alanine	231	no	4.3×10^5
Alanine	231	yes	2.5×10^5
Alanine	201	no	12×10^6
Alanine	201	yes	9×10^5

rough estimate that the lifetime of alanine would be cut from over one billion years to less than 10^5 years in the presence of oxygen. Assuming that the degradation reaction is of the second order, an activation energy of about 27,000 cal. mol may be calculated.* In the presence of a catalyst, visible light could furnish this energy, and the lifetime of amino acids in such an environment might be very short. Many substances are sensitive to light; the degradation of porphyrin solutions exposed to air and visible light at room temperature is a particularly interesting example since, in a dark anaerobic environment, porphyrin is one of the most stable organic substances.

Other Occurrences of Organic Constituents

A description of all the organic substances that have been reported as occurring in fossils, sediments, and sedimentary rocks is not feasible here. A

* Conway, who also has studied the effect of oxygen on alanine, has arrived at similar results independently.

particularly valuable recent review is that of Keilin.[7] A few occurrences, however, will be mentioned as a sample of what has been found. Erdman, Marlett, and Hanson[8] have recently found alanine, glutamic acid, glycine, proline, and leucine in an Oligocene (about 30,000,000 years old) marine mud now buried 5000 feet below the surface of the earth. A small amount of aspartic acid was noted, and valine was also identified tentatively. Vallentyne[9] has found α- and β-carotenes in the Pleistocene deposit at Searles Lake, Calif. Barghoorn[10] has described some remarkable examples of preservation of cellulose. A number of organic compounds, including large amounts of succinic acid, have been found in Baltic amber[7] approximately 40,000,000 years old.

Quite striking was the pioneering work of Treibs,[11, 12] who found porphy-

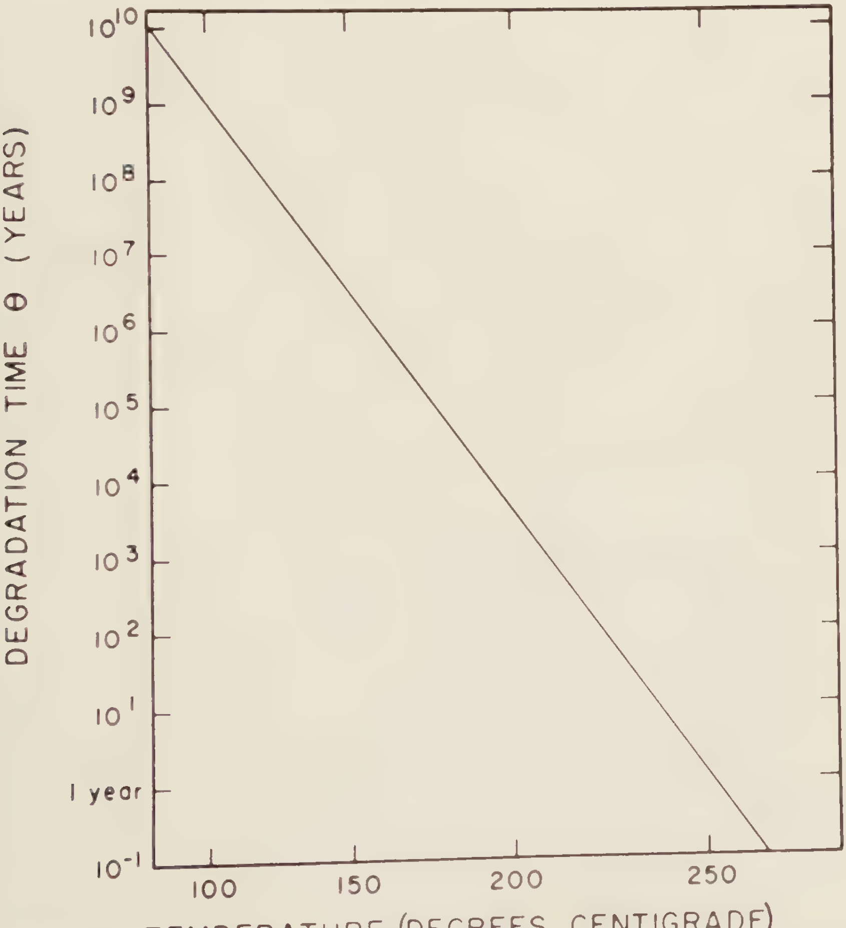

FIGURE 4. The thermal degradation of porphyrin conducted in an asphalt matrix. These are the results of experiments conducted by D. S. Montgomery[15] and are calculated for a first-order reaction with an energy of activation of 53,500 cal./mol.

rins in coal and shale and in petroleum as old as early Silurian (about 400,000,000 years). Studies of peats, lignins, and bituminous coals[13] have revealed a large number of compounds, including sugars, pentosans, fats, waxes, resins, and chlorophyll.

Because of their widespread occurrence, hydrocarbons and porphyrins in petroleum yield much evidence on the intrinsic stability of carbon compounds. The production of petroleum from old formations in which the temperature exceeds 100° C. is a striking reminder of the strength of the carbon-carbon and carbon-hydrogen bonds. Studies by McNab, Smith, and Betts[14] indicate a heat of activation for degradation of 58,000 cal. mol. This value is in accord with the natural occurrences. Montgomery[15] has made studies of the thermal stability of porphyrins in asphalts and finds an activation energy of degradation of 53,500 cal./mol. FIGURE 4 shows a plot of degradation time θ *versus* $1/T$ based on the equation $dc/dt = -kc$, where $k = Ae^{-E/RT}$, and $A = 10^{13}$ (a frequency factor), E = activation energy, R = gas constant = 1.986 cal./mol, T = absolute temperature. It can be seen that with favorable thermal histories porphyrins could be preserved for many billions of years.

Adventitious Effects

Finally, I shall discuss briefly the problem of adventitious contaminants. Organic substances move around in the crust of the earth. Petroleum migrates from source rocks to porous reservoir rocks. Free amino acids are found in soils and are soluble in ground water. Many cumulative events can occur in the course of millions of years. Hence the association of an organic substance with a sedimentary rock or fossil does not necessarily prove that the association has persisted since their original deposition. In fact, it is perhaps impossible to rule out some degree of adventitious contamination. However, in many cases one can establish strong circumstantial evidence that the organic substances associated with a given fossil are probably valid original substances.

An obvious criterion that must be satisfied is that the compound possess sufficient thermal stability to permit it to exist in the postulated environment. If, for instance, an amino acid were found associated with a fossil which, on geologic evidence, had long experienced a temperature of 200° C., the association clearly would be adventitious. With each fossil it is desirable to consider carefully all factors of burial history and environment and to examine the physical condition and mineralogy of the object.

A more positive line of evidence is to show that adsorption can be eliminated as the explanation for the presence of a substance. This can be achieved in the case of calcareous shells. Precipitating $CaCO_3$ partially adsorbs aspartic and glutamic acid, but it has virtually no affinity for neutral amino acids such as alanine. Actually, alanine, a neutral substance, is generally present in higher proportion than is glutamic acid or aspartic acid. Furthermore, in processing fossil shells, the outer 10 per cent of the shell may be dissolved and discarded. One can show a consistent pattern independent of environment by examining a number of fossils of the same species taken from a variety of localities.

Paleobiochemistry is an area for effort in research that holds many potentialities for unlocking nature's secrets. Information about many great events in the origin and evolution of life lies buried in rocks and fossils that wait, as they have waited for millions and billions of years, for man to seek them.

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ELECTROLYTE REQUIREMENTS OF PROTISTS AND ARCHEOMETABOLISM

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Rubey reasons that the ocean was salty and substantially unchanged throughout geological time and that the increment of salts deposited by leaching of the land was counterbalanced by an increase in the amount of water.[1] Consequently, if we assume that life began in the sea, electrolytes were very much a part of the environment for primordial life. The occurrence of inorganic electrolytes as essential constituents of organisms might be construed as the indelible imprint of the sea, as Macallum[2] regarded the pattern of salts in the body fluids of metazoa. Unfortunately, we are densely ignorant when we consider the question: to which part of the cell are electrolytes indispensable?

Metazoan metabolism supplies further incentives for work on the fundamentals of electrolyte requirements. Movements of Na^+ and K^+ characterize nervous activity;[3] what mode of intracellular communication preceded this development? To trace the physical basis of thought to its beginnings obviously requires a stupendous knowledge of the nature of life; less ambitiously, it demands an understanding of the mode of action of hormones. Certain steroid hormones drastically influence electrolyte relations in vertebrates; where are the protistan forerunners—the *Anlagen*—of these systems?

Our bias, directed by drudgery in the pursuit of indispensable trace elements and growth factors that lie well beyond the limits of sensitivity of conventional chemical analysis, makes us wonder at the current preoccupation with those quantitatively most abundant linear macromolecules, the nucleic acids and fibrous proteins that are invariant cell constituents, and with such conspicuous molecules as adenosine triphosphate (ATP). Investigation of the role of quantitatively minute invariants can be postponed only at the peril of impeding an increase in our knowledge of these macroconstituents. We are inclined to look upon certain inorganic electrolytes as indispensable to life and as capable of revealing much about its nature. While nucleic acids and associated protein may well be the repositories of genetic information, *how* is this information communicated to the effectors of the cell? We find it difficult to conceive of genetic information as merely a punched tape. The means of communication are part of this information, and the problem of communication is inseparable from that of the storage and duplication of information. In short, experiments designed to trace the ancestry of neurohumoral mechanisms, in which electrolytes play an important role, should bring one close to what the French call the "secret of life."

Some components of the original cell machine must have composed the substratum for the evolution of the neurohumoral systems that underlie thought, as closely as any reaction chains can be said to do this. The narrower problem considered here is: Can the electrolyte requirements of extant organisms provide clues to the origin of these systems?

It is not known how a hormone functions at the cellular level;[4, 5] correspondingly, we have almost no information on the equivalent in protists of the neurochemical systems in metazoa. Clearly, new research tools are needed, and we believe that protists might serve this purpose.

Before investing effort in elucidating the mode of action of electrolytes, envisioning them as archaic prerequisites for life, it is well to be assured that their assumed fundamental essentiality is borne out by their being universal nutritional requirements, apart from the gross use of electrolytes as osmoregulatory agents. The picture is reassuring. First, there is the universality of the K and Mg requirements.

Fresh-water blue-green algae need Na and K;[6] there can be little doubt that the blue-greens are a very conservative group. It is not known whether land bacteria require Na or halogens. Since autoclavable polyethylene culture vessels and chemical equipment are becoming available, it should soon be practicable to purify nutrient chemicals adqeuately; electrolytes are leached out of glass too easily. The sporadic occurrence of chlorine in antibiotics of bacterial origin (chlortetracycline and chloramphenicol, for example) hints that chlorine is essential for bacteria. This element is an essential micronutrient for higher plants.[7] The many reports of the use of NaCl as a fertilizer favor the likelihood that Na also will prove to be essential. Nothing is known about electrolyte requirements in fresh-water or soil protists other than bacteria.

The "why" of salt requirements in vertebrates is almost wholly obscure, aside from the connection of salt with nerve conduction and certain steroid hormones, and the occurrence of HCl in gastric fluid.[8] Thanks to our knowledge of thyroxine, the essentiality of iodine for vertebrates is recognized; the sporadic occurrence of iodotyrosine compounds in sponges and coelenterates might otherwise be dismissed as a biochemical freak. Perhaps the situation is similar for bromine, which was first known biologically in the molluscan product Tyrian purple (a brom-indigo); bromine may be an essential for vertebrates.[9, 10]

It is good strategy to study a function in a situation in which it is displayed in a specialized way on a large scale. To study locomotion, for example, one investigates muscle, not amoeba; to study fermentation, one investigates yeast. Which organisms are best for work on an electrolyte? Marine algae and protozoa have conspicuous salt requirements; some of the strains in pure culture seem to be exceptionally favorable material. One of the extreme forms is the green brine-flagellate *Dunaliella salina*, which can grow in tenfold concentrated sea-water or saturated NaCl.[11]

Some years ago we attempted to learn whether the tetraethylammonium ion spared the Na requirement of *Dunaliella*; we were inspired by the report[12] that the conductivity of certain frog nerves, placed in Na-free solutions, was restored by tetraethylammonium. The results were inconclusive. However, these experiments did show us that there was need for a metabolically inert, pure, low-molecular nonelectrolyte to serve to satisfy any nonspecific osmotic requirements. Metabolically inert, low-molecular cationic, anionic, and zwitterionic electrolytes were also required to satisfy any nonspecific need for an electrolyte and, for a brine alga and a salt-requiring bacterium, these spared Na. Our results also indicated that while triethanolamine and tetraethyl-

ammonium chloride did not spare Na for *Dunaliella*, the presence of pentaerythritol, as a presumable inert means of supplying additional osmotic pressure, reduced the Na requirement to 10 per cent of the minimal requirement in our standard medium; that is, from 5 per cent to 0.5 per cent. For some reason sugars inhibited growth. It was reported recently that the conductivity of another type of frog nerve kept in a Na-free solution was restored by guanidine chloride.[13] This has special interest, because a guanidine-like ion may be responsible for the maintenance of irritability in *Nitella* cells washed with distilled water.[14] Consequently, we have formulated a new working hypothesis; namely, that the replacement or sparing of Na (and perhaps K) may require a combination of organic cations. A number of years ago, in collaboration with Herman Ziffer of Mount Sinai Hospital, New York, N. Y., we attempted to ascertain whether the heightened need for Na in adrenalectomized rats could be met, at least in part, with such bulky, strong organic bases as tetraethylammonium but, owing to a succession of mischances, the experiments were inconclusive. We are unaware of any published work describing such attempts.

Much information on the mode of action of vitamins has come from studies on compounds that spare or replace them. If a vitamin participates in synthesis, and an organism that needs an exogenous source of this vitamin is supplied with the finished products of these syntheses, the vitamin may, in effect, be bypassed. The search for substitutes for the NaCl requirement of *Dunaliella* may be similar in principle. It is conceivable that, as organisms evolved, they elaborated organic substitutes for inorganic electrolytes. Where this seems to have happened, the osmoregulatory effect of electrolytes appears to be the function concerned. Thus, sharks use urea to maintain the isotonicity of their blood with sea water, and perhaps crustaceans use amino acids for the same purpose.[15, 16] This phenomenon suggests that part of the difficulty in demonstrating electrolyte requirements in nonmarine organisms may stem from the evolutionary development of electrolyte-bypassing factors as part of the evolution toward the fixity of the *milieu intérieur*, and that a trace requirement for electrolyte represents the least easily bypassable, the most indispensable—in short, the most primitive function of an electrolyte. At this point, where the problem is to demonstrate trace requirements, one might invoke another principle that originated in microbiology and that has been widely applied in biochemistry; namely, the use of inhibitors to increase the need for a metabolite and thus make it easier to demonstrate. The sulfa drugs provide the classic example. They were used first to demonstrate that *p*-aminobenzoic acid was a metabolite, and then in the demonstration that vitamin B_{12} was a product of a synthetic system in which *p*-aminobenzoic acid played a part. There are a few indications in the literature that this technique may be applied to the study of electrolyte requirements. *N*-Amylcarbamate is competitive with Na in constant ratio for the maintenance of the conductivity of nerve tissue.[17]

Competition with Na by derivatives of guanidine was noted by Larramendi, Lorente de Nó, and Vidal.[13] We have encountered exaggeration of the K requirement of bacteria by triethanolamine[18] in unpublished work with bacilli and algae. No reports have yet appeared on whether this holds true for pro-

tozoa and metazoa. The report that 2,4-diaminobutyric acid acts as a competitor for K in tissues[19] seems not to have been applied to protists as yet. Competition between K and Na is well known.

There may be an exception to our general ignorance of the action of hormones at the cellular level—if a yeast "cell" is functionally akin to a metazoan cell. Conway[2,p2] reports that deoxycortisone inhibits the uptake of K and the release of Na by yeast so grown that it had accumulated Na, and that compound E stimulates the uptake of K by yeast. This might be the basis of a microbiological test for hormones that influence electrolytes. Since animals that are deficient in corticoid hormones must be given extra Na, Na-retaining hormones are, in effect, Na-sparing factors. Steroid requirements are not uncommon in protists, for none is a steroid hormone active. Indeed, it is not even known whether steroid hormones occur in protists. Conway's observation thus is an isolated one; its extension to other hormones and protists might support his idea that regulation of permeability was the original function of hormones.

To investigate any possible relation between the electrolyte requirements of marine organisms and the postulated archaic, persistent, and unknown functions of electrolytes in all present organisms is to raise the question of *which* marine organisms to study, keeping in mind that lipid-soluble nutrients such as steroids must be taken into account. There is no evidence that brine flagellates such as *Dunaliella salina* are permeable to steroids. The probability that they are so seems slight, because *Dunaliella* so far has resisted all efforts to grow it in the dark. This may indicate an impermeability to energy-furnishing nutrients and, perhaps, to most other organic nutrients as well. There are marine protists that are able to absorb water-insoluble materials; these are the particle-ingesting (phagotrophic) protozoa. Many of these are photosynthetic flagellates. One such fresh-water flagellate, *Ochromonas malhamensis*, is used widely in the assay of vitamin B_{12}, because the specificity of its requirement of this nutrient is identical, by present indications, with that of vertebrates;[20] it seems to match the chick in ability to utilize vitamin B_{12} in crude natural materials,[21] perhaps because of its phagotrophy. For this and other reasons of comparative biochemistry, flagellates such as chrysomonads might be closer to the metozoa than any other protists now available in pure culture, and they might be favorable tools for use in the effort to find *Anlagen* of metazoan hormones. Certainly, a protist of this kind would be needed if electrolyte-sparing factors were bulky, lipid-soluble, or poorly soluble in aqueous and nonaqueous solvents alike. Since *O. malhamensis* is a fresh-water form, its requirements for monovalent electrolytes other than K may be minute and demonstrable only with the combined aid of rigorously purified chemicals and the aforementioned antagonists of electrolytes.

Another approach to this problem has been made. The bromine requirement in mice and chicks noted previously[9, 10] was brought to light by the analysis of the nutritional supplements that were required to overcome the depression of growth caused by feeding thyroactive materials. We have been studying the greatly enhanced nutritional requirements of *O. malhamensis* grown at incubator temperatures above the usual optimum. We are looking for parallels with the fever-heightened nutritional requirements in metazoa. Heightened

temperature, in fraying the biochemical fabric of the organism, brings to light otherwise poorly accessible metabolic chains. The appearance of electrolyte requirements as "temperature factors" would support the idea that bromine and other characteristically marine, but biochemically unfamiliar, electrolytes are prerequisites for life. The effectiveness of the temperature approach may be gauged from the fact that the vitamin B_{12} requirement of *O. malhamensis* is so augmented by elevated temperature ($\sim$ five hundredfold and, in animals fed thyroactive materials, $\sim$ fourfold) that, had vitamin B_{12} and cobalt been unknown as growth factors, they easily could have been identified in the role of "temperature factors." These results are being prepared for publication.

Marine phagotrophic chyrsomonads are common, but none are yet in pure culture. However, the increasing success in cultivating delicate marine dinoflagellates, diatoms, obligately photosynthetic chrysomonads, and several other kinds of marine plankton protists[22, 23] encourages the hope that the cultivation of highly phagotrophic marine chrysomonads may be achieved soon. These plants might well be the most favorable organisms of all for studying the nature of electrolyte requirements.

Before ending this account of protistan tools, present and potential, for electrolyte studies, mention should be made of the red halophiles, which are a group of organisms similar to the myxobacteria. Most of them need at least 14 per cent NaCl to grow. The sliminess of these extreme halophiles and of several colorless halophilic bacteria resides in an extracellular layer of deoxyribonuclease (DNA), as shown by direct isolation and removal of the slime layer with it.[24]

One is tempted, on the basis of the DNA layer of halophiles and the extreme sensitivity of DNA to variations in ionic strength, to ascribe to electrolytes a role in controlling the ionization and degree of hydrogen bonding of DNA.[25] Perhaps the specificity of electrolytes originally depended on different spatial constraints along the closely packed DNA helix and its associated protein. The DNA layer of the halophiles suggests that the converse may hold, and that DNA originally controlled directly the water and electrolyte equilibrium of the organism. Intimacy with the environment could hardly go further; no function save that of reproduction itself could be more vital. Much of evolution, then, might have been, and still can be, the elaboration of controls interposed between DNA and agents for homeostatic responses to the environment. DNA was thus protected, ever more effectively, from fluctuations in the environment, which was all the better for genetic stability. The physical state of extracted DNA (a polyelectrolyte) is highly sensitive to variations in the ionic strength of the medium. NaCl, for example, protects it against heat denaturation, and divalent cations such as Ca^{++} and Mg^{++} stabilize it much more efficiently than do monovalent ions.[25] Exactly how electrolytes affect the shape of DNA and vital proteins (and thus, necessarily, their function) presents a problem for future investigation. The present-day electrolyte requirements are so many clues, could we but read them, to the primordial functions of DNA or whatever substance served as the original center of information and communication.

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SPECULATIONS ON THE ORIGINS AND EVOLUTION OF PHOTOSYNTHESIS

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Introduction

There is a constant urge in man to seek beginnings. In biology, the beginning means the origin of life. Nearly a century ago Pasteur performed decisive experiments that led to the development of the important theory that all life develops from living cells. However, Pasteur himself realized that this did not hold the answer to the origin of life.[1] Now we are a century wiser. A number of young sciences such as biochemistry, geochemistry, nuclear chemistry, and solid-state physics already have added important generalizations to our knowledge. Instead of discussing the question of the origin of life solely from a general philosophical point of view, we now have the knowledge to seek experimental approaches to this question, not only in terms of life on this planet, but on other planets and planetary systems as well.

I have been asked to discuss the possible origins of photosynthesis in relation to hypotheses on the origin of life. By way of introduction I shall first summarize recent proposals on the origin of life and photosynthesis, and outline briefly the speculations that I wish to present. I shall then proceed to discuss these speculations in detail.

More recent proposals concerning the origin of life assume the following sequence of events.[2-5] First, a variety of complex organic molecules accumulated in the sea due to the action of ultraviolet light and electrical discharges on a mixture of ammonia, water, hydrogen, and methane or the oxides of carbon. Sufficient time then elapsed to allow the formation of a heterotrophic organization of units of protoplasm that used the organic compounds around them for growth. Clay minerals could have served as inorganic catalysts. Gradually, as these organic compounds were depleted, photosynthesis occurred, with chlorophyll or related compounds as the photosynthetic pigment. Then came the development of biosynthetic chains that originated from the functional end products as we know them today and, working backward, the production of genes and enzymes that would provide the precursor steps for the end product. The formation of a living entity was a one-way process. Once the complex organic materials of the environment were exhausted, new life could be started only from existing cells.

Today I wish to present speculations that differ in a number of ways from the above hypotheses. I shall propose that the first organization of preprotoplasm would be a primitive energy-conversion unit that could perform the elementary processes of photosynthesis and respiration; that this unit originated from some common minerals; that the minerals that contain metal ions served both as coordinating templates and catalysts for various reactions, and that around this unit were formed organic molecules that gradually became organized into units of ever-increasing complexity. Gradually, biosynthetic chains de-

veloped in a stepwise fashion, using small molecules to make molecules of ever-increasing complexity. The metal catalysts of minerals became modified into the metalloenzymes; in these new complexes the same metals would now act as more efficient catalysts.

The experimental method whereby it is proposed to find the evolutionary precursors of protoplasm is to examine present-day biochemical reactions in protoplasm and seek to relate them to reactions that may have occurred and may still occur in the minerals around us. On the basis of these speculations one might expect to find the elementary processes of evolution currently going on; there should be biological "niches" in which some of these intermediary evolutionary stages to the primitive cell would be preserved.

The Two Basic Requirements of Protoplasm

There are two basic requirements for the formation and functioning of any kind of protoplasm that we can imagine. One requirement is that protoplasm be primarily a liquid. A protoplasm based on either the solid or gaseous state would not be feasible; the solid state does not admit of sufficient plasticity and speed of diffusion, and the gaseous state does not impart sufficient rigidity. A second requirement is a source of energy that is relatively constant and unfailing; namely, the light of the star to which the planet belongs.* On the earth, the predominant liquid is, of course, water, and the energy which makes life possible is that of sunlight.†

Water is not only the medium of protoplasm, but it so happens that water enters intimately into the primary reaction of photosynthesis and the ultimate reaction of respiration. That is, water takes part chemically in the over-all changes of protoplasm. These over-all changes may be summarized by two reactions:

$$2H_2O + nh\nu \xrightarrow[\text{photosynthesis}]{\text{chlorophyll}} O_2 + 4H^+ + 4(e)^- \qquad (1)$$

$$O_2 + 4H^+ + 4(e)^- \xrightarrow[\text{respiration}]{\text{heme}} 2H_2O + \text{maintenance, duplication, heat, etc.} \qquad (2)$$

The first reaction represents the decomposition of water into oxygen, protons, and electrons by photons ($nh\nu$) from the sun. The $(e)^-$ of this reaction may be considered as electrons the high potential energy of which is stored in the form of electron-pair bonds of such organic compounds as starch. This reaction requires the photocatalyst chlorophyll, a green pigment that belongs to the family of compounds called the porphyrins. The reaction in the reverse direc-

* Instead of sunlight one might postulate some long lived radioactive process as a source of energy; such a source might be available for "life" in stars that have become cold. Life based on a chemiautotrophic existence would be possible under restricted conditions and for a limited time.[7]

† The light energy absorbed by the earth is approximately equal to the light energy re-emitted by it. The over all interaction may be considered as the absorption of photons of higher frequency and the re-emission into space of a greater number of photons of lower frequency (that is, heat radiation).

tion expresses an oxidation in which the electrons of high potential energy combine with protons and O_2 to form water where the electrons have a lower potential energy. The reaction of oxidation is catalyzed by an enzyme that has the pigment heme or iron porphyrin as prosthetic group. This release of free energy during oxidation is used by protoplasm to maintain itself and to make more of itself from the components of its environment. Thus, water is not only the medium of protoplasm, but is involved chemically in the fundamental processes of photosynthesis and respiration of protoplasm.[9]

As we have already noted, an essential requirement of protoplasm on any planet is its liquid state; it may not be essential, however, that this liquid be decomposed by light as part of an energy-storage process and be reformed again to release energy, but it is certainly convenient. Some other constituent in the liquid might serve this photosynthesis-respiration function.

If one considers what liquids might serve for the medium of protoplasm at different temperatures and pressures, one is limited in choice. One is limited more drastically if the additional requirement is added that the liquid be decomposable by light.

If a planet is relatively closer to its star, its temperature will be higher (500° to 1500° K.), and more intense light would be available for photodecomposition, but the quality of spectral emission would not differ appreciably from that radiated to more distant planets. A chemical bond is broken more readily by by photodecomposition if the photon has a higher frequency and the temperature of the environment is higher. If the planet is farther from the star, its temperature will be lower (100° to 200° K.), and the limitation of photosynthesis would be the intensity of light received. If comparison is made only on the basis of this difference in light intensity, it is conceivable that a protoplasm might have evolved more rapidly at a temperature of 500° to 1000° K. than at our own temperature, and that at 100° to 200° K. the evolution of protoplasm might have been even slower than that of our own.

The liquid proposed for a protoplasm at a particular temperature need not be one that is of great abundance. For example, water represents only 0.025 per cent of the earth's mass. At 500° to 1500° K., one possibility is a liquid composed of Si—O or Si—S bonds; studies on lavas might provide clues as to the composition of liquids at these temperatures. At 200° to 250° K. ammonia appears to be a reasonable choice for a liquid, both from considerations of photosynthesis and of ionization.[10] For example, if we compare the photodecomposition of water with that of NH_3 we see interesting resemblances.

$$\begin{aligned} 2H_2O &\rightleftharpoons 4H^+ + 4(e)^- + O_2 \\ 2NH_3 &\rightleftharpoons 2H^+ + 2(e)^- + NH_2 - NH_2 \end{aligned} \tag{3}$$

One may also compare the ionization process of water with that of NH_3.

$$\begin{aligned} 2H_2O &\rightleftharpoons H_3O^+ + OH^- \\ 2NH_3 &\rightleftharpoons NH_4^+ + NH_2^- \end{aligned} \tag{4}$$

It is interesting to observe that our protoplasm, in all its complexity, uses

chemical reactions that have a maximum chemical intensity of only about 1.2 volts. If we compare this intensity with the intensities of other chemistries we shall see how small it is. The potentials involved in the nuclear chemistries of the stars are expressed in millions of electron volts. The intensity of chemical reactions on earth is usually no greater than 6 electron volts, as for example in the reaction between the elements Li and F. This is so because our "inorganic chemistry" is concerned mainly with the reactions of the loosest or outermost orbital electrons. The chemistry of protoplasm often is concerned with reactions of certain of the still looser electrons of organic molecules.

Because the reactions of protoplasm occur in an aqueous medium, the chemistry of protoplasm can operate only within the narrow limits of 1.2 volts; that is, between the potential of the hydrogen electrode and that of the oxygen electrode. The limits are imposed by the fact that a reducing intensity greater than that of the hydrogen electrode would liberate H_2 from water, and an oxidizing intensity greater than that of the oxygen electrode would liberate O_2 from water. For example, the ΔE calculated for the oxidation of pyruvate to acetate is about 1.4 volts. It is found, however, that this oxidation in protoplasm proceeds in two steps; first, the transfer of electrons to reduce a DPN molecule (diphosphopyridine nucleotide) ($\Delta E = 1.1$ volts), and, second, the formation of a high-energy —S or —PO_4 bond. In neither reaction is the chemical potential ΔE of 1.2 volts exceeded.

From some of the recent data on photosynthesis[11, 12] one may assume that DPN is reduced readily, so that a maximum chemical intensity ΔE of 1.1 to 1.2 volts could be generated during the photodecomposition of water. The maximum reducing ability of an electron in an aqueous medium, is theoretically, 1.2 volts. Perhaps studies on photosynthesis eventually may show that plants have found a way to decompose water so that an electron $(e)^-$ of ΔE 1.2 volts is produced per photon absorbed.

Photosynthesis in Primitive Atmospheres

In order to postulate a scheme of primitive photosynthesis it would seem necessary to know the conditions of the primitive atmosphere at the time the earth was formed. There are two hypotheses on the composition of the atmosphere, one proposed by Urey[1] and the other suggested by Rubey.[13] Both hypotheses assume that the earth was formed by an aggregation of solid particles, the planetesimals. The components of the earth's atmosphere and the water of the oceans would have been formed by a gradual release from the earth's interior of the gases originally occluded or chemically combined in the solid particles.

Urey has proposed that the original atmosphere was highly reducing, all of the components being combined with hydrogen. The gases were H_2, CH_4, NH_3, and H_2O. The gases were ionized by the action of ultraviolet light to radicals which condensed to form complex organic molecules that dissolved in the oceans (Miller's experiment with a similar gas mixture, in which radicals were created with electric sparks, resulted in the formation of organic materials, among which were identified some simple amino acids).[5] Water in the atmosphere would be decomposed by ultraviolet (UV) light to form the radicals H

and OH. The OH radicals would be used to oxidize NH_3 and CH_4. The H_2 would gradually escape from the earth and the composition of the atmosphere would slowly become less reducing. O_2 would appear and the UV light acting on O_2 would result in the formation of ozone. The ozone would act as a barrier to the penetration of UV light to the earth's surface. Thus, Urey would start with a reducing atmosphere, and gradually this atmosphere would be converted to an oxidizing one.

Rubey[13] has proposed, on the basis of geochemical calculations, that the atmosphere may have consisted primarily of N_2, CO_2, and H_2O. Under such conditions the formation of a complex organic mixture by UV irradiation of the atmosphere would be more difficult to imagine than if the atmsophere had contained NH_3, CH_4, H_2, and H_2O.

Both hypotheses agree that little or no free O_2 was present. This conclusion is supported by calculations that indicate that if photosynthesis of plants were to cease, all the free O_2 of our atmosphere would be used up in about 2000 years.[14]

The photodecomposition of water may occur directly or indirectly. A photon of UV light ($\lambda < 3200$ Å.) has sufficient energy to break a bond of a water molecule directly into two radicals, a reductant H and an oxidant OH.

$$\mathrm{HOH} \rightarrow \mathrm{H} + \mathrm{OH} \qquad \Delta F = +56{,}000 \text{ cal.} \tag{5}$$

A photon of visible light has less energy. It cannot split water directly into radicals. Rather, interactions with other atoms or molecules are required to decompose a water molecule, not into radicals, but into some unknown compounds that at present may best be written as reductant (H-x) and oxidant (y-OH).

$$\mathrm{HOH} \rightarrow (\mathrm{H}\text{-}x) + (y\text{-}\mathrm{OH}) \tag{6}$$

Here x and y are the atoms of molecules (not necessarily the same molecules) that form bonds with H and OH.

The photosynthetic bacteria and higher plants all appear to have the ability to decompose water by the action of visible light, a generalization first proposed by Van Niel.[15] Let us review this reaction briefly. We shall see that once the mechanism for photodecomposition of water was discovered by protoplasm, it would have been possible for organisms to have started, either with a reducing atmosphere of the type proposed by Urey, or with an atmosphere of the type proposed by Rubey, and have converted the atmosphere eventually into an oxidative one.

In an inorganic medium, photosynthesis (autotrophic) consists of the decomposition of water into the reductant (H-x) and the oxidant (y-OH). Two molecules of reductant are required to reduce DPN to DPNH. Two DPNH molecules then reduce CO_2 indirectly to the level of carbohydrates; that is, (CH_2O) by way of the Calvin cycle. In addition to the DPNH, some adenosine triphosphate (ATP) is required for CO_2 fixation, and this might come from oxidative phosphorylation of DPNH by the oxidant (y-OH).

Also, under aerobic or anaerobic conditions, in the absence of excess reducing

$$\begin{array}{llcll}
 & H_2 & & (CH_2O) & \\
 & \Big\uparrow \text{Fe, Mo} & & \Big\uparrow CH_4 & \left.\right\}\text{Heterotrophic-anaerobic atmosphere} \\
4\,HOH \rightarrow & 4(H\text{-}x) & + & 4(y\text{-}OH) & \\
 & \Big\downarrow 2\,DPN^+ & & \Big\downarrow Mn & \\
 & 2\,DPNH & & O_2 + H_2O & \left.\right\}\text{Autotrophic-aerobic or anaerobic atmosphere} \\
 & \Big\downarrow CO_2 & & & \\
 & (CH_2O) & & &
\end{array}$$

FIGURE 1. The products that may result from the photodecomposition of water under aerobic conditions, or under anaerobic conditions of a nonreducing atmosphere (that is, a Rubey atmosphere), are O_2 and carbohydrates. Under the heterotrophic-anaerobic conditions of a reducing atmosphere (that is, a Urey atmosphere) the products would be H_2 and carbohydrates.

substances in the medium, the oxidant (y-OH) is converted to O_2. There is some evidence that Mn is involved in this step or, possibly, in a peroxide-decomposition step.[16] Thus, the usual photosynthesis leads to the formation of carbohydrate and O_2, and these are the products that would result from a Rubey atmosphere.

Under heterotrophic, anaerobic conditions, in a medium that contained excess reducing substances such as malate, glutamate, or substances that might have been formed in a Urey atmosphere and concentrated in the ocean, the organism would require an oxidant for the release of energy. Its metabolism would be limited by the amount of oxidant that it could obtain. Thus, the oxidant (y-OH) produced in photosynthesis would be exhausted rapidly and would never be converted to the O_2 stage.

For example, photosynthetic purple nonsulfur bacteria are grown under conditions of excess reductant as substrate in an anaerobic atmosphere. They create oxidizing conditions by photosynthesis and use the oxidant (y-OH) for oxidation. In purple sulfur bacteria that perform photosynthesis in an atmosphere that contains N_2, CO_2, and H_2S, the (y-OH) reacts in part to oxidize H_2S to elemental sulfur.[17, 18] Not only may the reducing organic compounds of the medium be converted to more oxidizable components by the oxidant (y-OH), but oxidizing conditions may be aided by the release of H_2 to the atmosphere, perhaps by direct conversion of (H-x) to H_2.

Under anaerobic conditions in a highly reducing atmosphere of CH_4, NH_3 and H_2 (that is, under the conditions of the Urey atmosphere) photosynthesis based on the decomposition of water might have proceeded as shown in the upper portion of FIGURE 1. The H-x might have been converted to H_2, which then could escape into the atmosphere and diffuse slowly from the earth into outer space. In this way the atmosphere could have become more aerobic (the hydrogenase enzyme that catalyzes the release of H_2 appears to contain the metals Fe and Mo, and is known to be present in many bacteria and algae). The CH_4 might have been oxidized by the oxidant, (y-OH), to (CH_2O), and thus carbohydrates could be formed, not from CO_2, but from methane.

Another way by which anaerobic photosynthetic bacteria might have accelerated the loss of H_2 from a Urey atmosphere is suggested by studies of the fixation of N_2 to NH_3[19] and the reverse process, denitrification. The dentrification could have converted NH_3 to N_2 and H_2, a process that requires only a small change in free energy. After H_2 had disappeared from the atmosphere the photosynthetic bacteria could have used (H-x) to fix N_2 to NH_3 as they appear to do at present.

Thus, the process of photodecomposition of water, beginning on the primitive earth with the conditions postulated either by Rubey or Urey, eventually would lead to the oxidizing atmosphere that we have at present.

If a unit of mineral origin had the property of being able to decompose water by the action of visible light into oxidant and reductant, either a Urey or Rubey atmosphere would have been suitable as a point of origin for the synthesis of preprotoplasm.

A Biochemical Approach to Studies of Evolution

The biosynthetic chain. In studies of evolution we attempt to find certain attributes of present-day protoplasm that we can trace back embryologically, phylogenetically, or paleontologically. With the development of biochemistry came the discovery that all cells are built on a similar basic plan, the common denominator being a series of biosynthetic chains that consist of genes, enzymes, intermediate substrates, and the end products of the chains. On the basis of this generalization of biochemistry, Horowitz[3] has proposed that the biosynthetic chain represents an evolutionary development. We think that this is an excellent idea. However, Horowitz has proposed further that the functional product at the end of the biosynthetic chain was produced first, and that mutations occurred one at a time in a direction opposite from that in which synthesis to the functional product now proceeds. For example, on the basis of this latter hypothesis the biosynthetic chain of chlorophyll would first begin with the functional end product, chlorophyll and, later, by a series of gene mutations, would form the precursor steps down to the elementary building blocks, acetic acid and glycine. It is this second proposal of Horowitz which we would alter.

The pathway of heme and chlorophyll synthesis by way of the common precursor, protoporphyrin, suggests an evolutionary pathway which we believe is interpreted better on the basis of a progressive development in the direction of the present-day functional end products rather than in the reverse direction. FIGURES 2 and 3 present a scheme of biosynthesis to chlorophyll. The building blocks of heme and chlorophyll are glycine and succinate.[20, 21] For the formation of the first stable intermediate, ∂-amino levulinate, there is required a functioning citric acid cycle coupled to an electron transfer system to O_2; in addition, pyridoxal phosphate is also necessary. Specific enzymes are now known that will convert ∂-amino levulinate through the monopyrrole, porphobilinogen, to the colorless uroporphyrinogen, and coproporphyrinogen to the red pigment protoporphyrin. The insertion of Fe into protoporphyrin results in the formation of the oxidative heme catalysts. The insertion of Mg into protoporphyrin leads through several modified Mg porphyrins to chlorophyll.

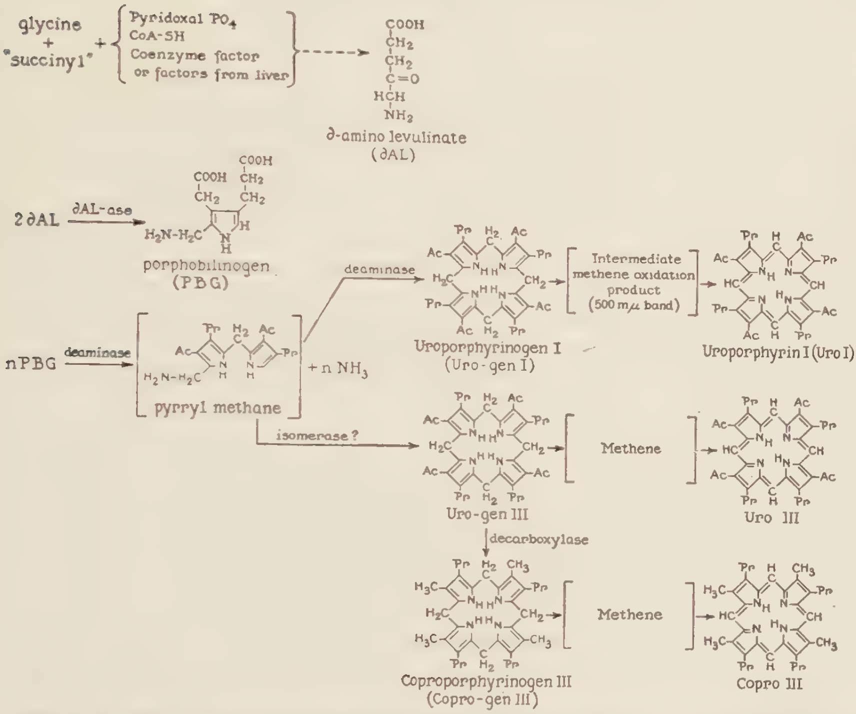

FIGURE 2. Substrates and enzymes that lead to protoporphyrin, as determined from studies on the red blood cell.

Let us see how we might picture, from an evolutionary point of view, the synthesis of these important pigments on the basis of the known properties of the intermediates noted in the scheme. If the building blocks glycine and some active succinate compounds were formed, it is possible that ∂-amino levulinate might form.* Once ∂-amino levulinate has formed, it may condense with itself spontaneously in low yield to form porphobilinogen.[22]

Once porphobilinogen is formed it may condense spontaneously to form, among other products, some uroporphyrin III in the presence of an oxidant. Uroporphyrin is a highly colored red fluorescent pigment that could perform photosensitized reactions or could complex with metals such as Fe or Cu to form metalloporphyrins, which might catalyze oxidations, and so forth.

One might postulate that such products resulting from nonenzymic condensations of ∂-amino levulinate might have given the cell selective advantages; in time, mutations might have occurred to create these colored functional products by enzymic reactions. Progressive changes in the biosynthetic chain would be postulated to perform these functions with greater efficiency.

In general, it is necessary to assume that at some time each one of the intermediate compounds in the biochemical sequence became the end product of

* Glycine and succinate have been reported by Miller to be formed by spark discharge through an atmosphere of NH_3, H_2, CH_4, and H_2O. It is probable that such compounds could also be formed readily by a primitive type of photosynthesis (see below).

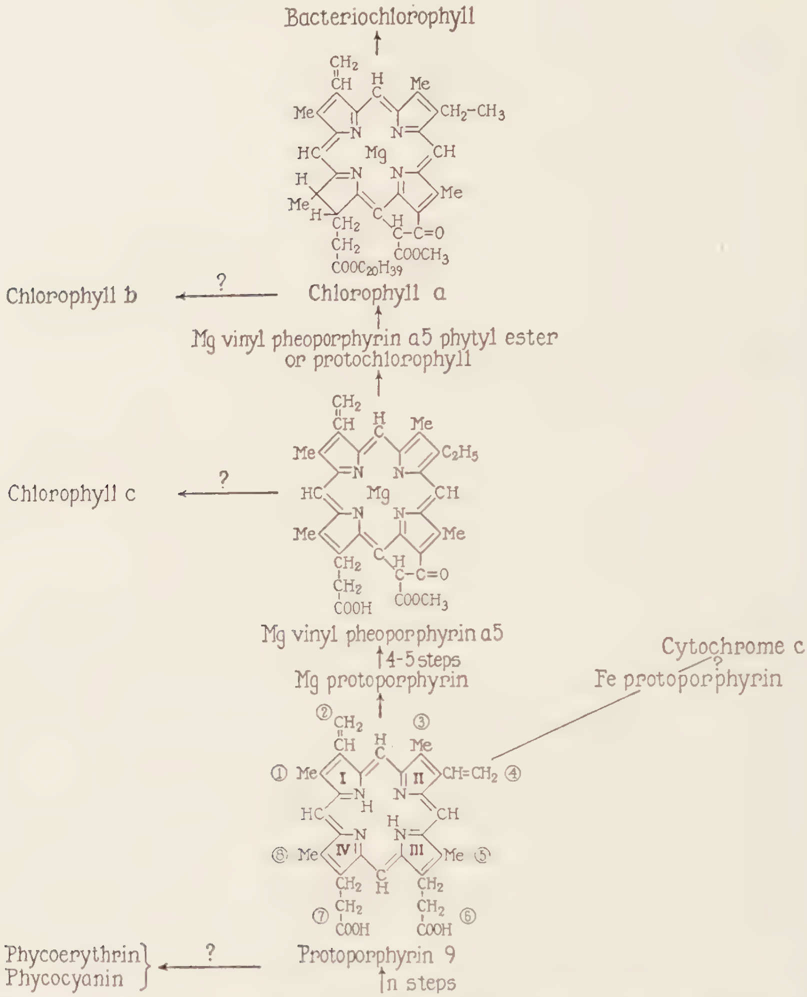

FIGURE 3. Substrates that lead from protoporphyrin to chlorophyll, as determined from studies on *Chlorella* mutants.

the sequence. As end product, such a compound might have had a function similar to that of its precursor in the biosynthetic chain. For example, Mg protoporphyrin at one time might have been the end product of the chain and might have served as photosynthetic pigment. When additional steps were added to the sequence, the original function of Mg protoporphyrin might have been lost, but the steps to form Mg protoporphyrin, which include the precursors, the specific genes and enzymes, could not have been lost, because Mg protoporphyrin now functioned as an intermediate.

According to this hypothesis, all of the colored compounds arising in the evolutionary sequence that leads eventually to chlorophyll might have had the

same function as chlorophyll itself; namely, that of photosynthesis. At present, no photosynthetic pigments except chlorophyll *a* and bacteriochlorophyll (B 890 mμ) are known. However, we know of pigments related to precursor pigments of the biosynthetic chain that take part in photosynthesis. For example, the phycocyanins and phycoerythrins of the red and blue-green algae absorb light and transfer the energy to chlorophyll *a*; these pigments are related to open-chain tetrapyrroles. A chlorophyll *c* that might have a similar function is related to Mg vinyl pheoporphyrin *a*5. Another pigment, protochlorophyll *a*, the immediate precursor of chlorophyll *a*, appears to be photoreduced by the action of light to chlorophyll *a*. The vestiges of photoactivity by these compounds are suggestive bits of evidence that precursor pigments to chlorophyll might have been functional end products serving for photosynthesis at earlier periods in evolution.

It does not seem reasonable to consider that, after many different evolutionary steps that finally gave rise to colored pigments, the properties of oxidation and photosynthesis were suddenly created. It seems more reasonable to consider that the functions of oxidation and photosynthesis were so fundamental that they were part of the first beginnings of protoplasm that arose from inorganic origins. The properties of the present-day heme, as will be discussed below, are essentially those of the iron atom, and they may be found in those of the inorganic iron compounds themselves. Also, perhaps, the property of chlorophyll might be represented by some colored inorganic compound.

The hypothesis that I propose is that one may trace back to inorganic origins the primary mechanisms for the functioning of protoplasm, and that the evolutionary process represents the progressive elaboration of mechanisms, by way of biosynthetic chains, for performing these functions in a more efficient manner.

Catalysis by inorganic metal ions and their relation to enzymes. To illustrate the hypothesis that one may trace back to inorganic origins the primary mechanisms for the functioning of protoplasm, let us consider inorganic iron, its properties, and its possible evolution into heme porphyrin enzymes.

The inorganic iron atom possesses at least four properties that find their counterpart in specific heme enzymes.* For example, the property of electron transfer by cytochrome C may be represented by the reversible oxidation of ferrous to ferric iron. The activity of cytochrome oxidase may be represented by the reaction of ferrous ions with O_2. The catalase and peroxidase properties are also possessed by inorganic ferrous and ferric iron. The same inorganic atom then can act in these four catalytic capacities. It is nonspecialized as far as these four properties go; its catalytic activities are rather weak as compared to those of the heme enzymes.

Let us examine, for comparison, the catalytic property of inorganic iron and that of catalase to see how iron compounds might have been modified during evolution to split H_2O_2 molecules to O_2 and water more efficiently. Inorganic ferrous iron has a very low catalase activity. Certain ferric hydroxides may

* Reversible O_2 transport as in hemoglobins, although not yet reported as a property of inorganic iron compounds, has been revealed in the simple cobatous complex $[Co(NH_3)_5 - O_2 - Co(NH_3)_5]Cl_4$.[23] The functioning of iron as a catalyst in hydrogenase reactions also should be noted; perhaps this function is related to the paramagnetic properties of the iron atom.

TABLE 1

BOND CONFIGURATIONS AROUND SOME METAL IONS

Ion	Coordination No.	Structural type
Na^+	6	Octahedral
K^+	6	Octahedral
Mg^{++}	6	Octahedral
Ca^{++}	6	Octahedral
Zn^{++}	4	Tetrahedral
$Mn^{2,3,4+}$	6	Octahedral
$Fe^{2,3+}$	6	Octahedral
$Co^{2,3+}$	6	Octrahedral
$Cu^{1,2+}$	4	Square planar Tetrahedral
$Mo^{5,6+}$	8	Dodecahedral

show a hundredfold increase in activity as compared to ferrous iron. When iron is incorporated into the protoporphyrin ring to form heme, the resulting molecule may show a thousandfold increase over the activity of inorganic iron. Now, when the heme molecule becomes attached to a specific protein, the catalase activity is enhanced about one million times. Thus, we see that by incorporating iron into a ring compound and then attaching this ring compound to a special protein, a selective activity has been attained; namely, that of splitting H_2O_2, and this activity has been enhanced a millionfold over that of the nonspecialized inorganic ferrous iron.

The examination of many enzymes is revealing more and more the intimate role of metals in enzyme catalysis.[24] The properties of the metal ions in proteins, are, in general, the properties of the inorganic metal ions themselves, as illustrated above for the case of iron. The inorganic metal ions could have served as catalysts in the inorganic mineral units around which protoplasm developed.

Why do the metal ions have catalytic properties? They are catalysts essentially because they can surround themselves and form coordinate bonds with a number of atoms or atom groups, as illustrated in TABLE 1. Thus, Mn, Fe, and Co can form coordination bonds with 6 atoms; Zn and Cu can form coordination bonds with 4 atoms; Mo with 8 atoms. In general, there are 4 ways in which metals may catalyze a reaction:

(1) The metal ion can bring together, in close apposition, atoms of different molecules and thus increase the concentration of the reacting molecules.

(2) The metal ion, acting as a Lewis acid,* can catalyze certain acid-catalyzed reactions at neutral *p*H. The metal ion, by virtue of its small size and multivalent positive charge, has an avidity for electrons and can distort the atom of a molecule that attaches to the metal ion. Perhaps the Zn atom in carboxypeptidase might serve in this way to catalyze the hydrolysis or group transfer of certain peptides.[25] Model reactions of metal-catalyzed systems are being studied in a number of laboratories. For example, Cu^{++} catalysis of

* A Lewis acid is a substance that has a vacant orbital that can accept an electron.

the hydrolysis of phenylalanylglycine amide at the amide and peptide bonds have been reported.[26]

(3) The metal ion may act as redox catalyst. Certain metal ions such as Fe, Co, and Cu have the property of acquiring or releasing one electron and thus acting as oxidation-reduction catalysts. In the reduced form they may bind certain atoms weakly; in the oxidized form they may bind certain atoms strongly, or vice versa. The same metal ion may function over different ranges of potential, depending on the complexes formed. A host of possibilities for catalysis is offered by such properties. For example, the production of high-energy phosphate bonds by way of the electron transport system of mitochondria appears to involve a mechanism of oxidation and reduction of metal ions that is directly coupled with high-energy phosphate bond formation.

(4) The metal ions may not only form a specific number of bonds, but certain bond directions are preferred with certain atoms. Thus, Zn and Cu ions both can coordinate with four atoms, but the spatially preferred bond directions are tetrahedral for a Zn complex and planar for a Cu complex. Perhaps, if certain molecules of more rigid structure can attach to the metal ion, a distortion of bonds might result, and such a mechanism might explain certain catalyses. The change from a tetrahedral to a planar configuration that might occur in the oxidation of copper might furnish a mechanism for breaking bonds.

Energy Unit of Mineral Origin as the Point of Inception of the Origin of Protoplasm

If we are to assume that protoplasm originated from some inorganic mineral-like materials, what are the necessary properties of such materials? I propose, as speculation, that the earliest unit around which any living entity arose was an energy-conversion unit. This unit of mineral origin would contain an organization of atoms that would serve as a photocatalyst, at first perhaps in the decomposition of water by UV radiation. Such a decomposition of water into radicals would be useful only if the radicals did not recombine immediately. Other atoms of the mineral in the immediate vicinity could absorb the radicals, thus increasing their lifetimes. Still other atoms, such as the metal ions, could serve as catalysts to orient and concentrate the radicals and reactants. On these surfaces, the synthesis of organic materials could occur more efficiently in contrast to a photosynthesis of the random kind that might occur also at this early time in the reducing atmosphere. Thus, a complex array of molecules would become concentrated and organized around the mineral photocatalyst.

Within a shorter or longer time, as the atmosphere became more oxidizing and UV light was filtered out, there would necessarily develop a mechanism of photodecomposition of water in which now not one light quantum of UV light, but at least two quanta of visible light would be required. The assumption of a rather elaborate organization is required for such a process to operate.

A simpler hypothesis to explain trapping the energy of light is that proposed

by Benson and Calvin.[27] They suggest that the photo-oxidation of ferrous phosphate might provide energy for the formation of a pyrophosphate bond, and that this low-grade phosphate bond energy might have served in many reactions akin to the functioning of ATP. A reaction of this kind might be useful in supplying energy for group-transfer reactions, but not for oxidation-reduction reactions. It might have preceeded the development of a mechanism for the photodecomposition of water or have been concomitant with it.

At present we can only speculate on the mechanisms for the photodecomposition of water that might have occurred in the presence of inorganic minerals. With increasing knowledge of the physics of photoconduction in the solid state we may hope eventually to know what to seek in terms of mechanisms of early photosynthesis.

To determine which properties of minerals one should attempt to study in terms of a photodecomposition of water, it would be well to know how present-day photosynthesis proceeds. Unfortunately we do not know the precise mechanisms as yet. We do know some of the requirements, however.

Let us summarize what we know and surmise about these requirements: A molecule, chlorophyll, that has an intense absorption in the visible region is used. The absorbed energy is then converted, apparently, to a uniform packet of energy that is equivalent to that of a photon of λ 6800 A. The chlorophylls are tightly packed in the chloroplasts, possibly in a concentration as high as 0.1 molal, which permits the transfer of light energy from chlorophyll *a* molecules to other chlorophyll *a* molecules by resonance transfer. The energy finally is imparted to some unknown acceptor. Assuming that one light quantum would liberate one molecule of reductant (H-*x*) of sufficiently negative potential, then 2 (H-*x*) might be used for the reduction of DPN^+ to DPNH (TABLE 1). The DPNH molecule serves to store energy in a reduced form that is not readily autoxidized by O_2, thus preventing a back reaction; i.e., a reaction between (H-*x*) and (*y*-OH) which would lead only to heat liberation. The (*y*-OH) is removed as O_2 in connection with Mn catalysis, which also prevents a back reaction, since no cytochrome oxidase is present in the chloroplasts to activate the O_2. The highly complex layering in chloroplasts might be considered to serve to concentrate certain molecules such as chlorophyll in specific layers and to facilitate separation of oxidant from reductant so that back reactions are avoided (the function of activating O_2 for respiration occurs in separate cytoplasmic bodies, the mitochondria).

What kind of mineral might have properties, similar to those of the chloroplast, that are used in photosynthesis? One of the minerals that might have such properties to a limited degree is an impure spinel[28] composed principally of magnetite ($FeO.Fe_2O_3$) and containing some sulfur.* Some of the divalent and trivalent iron atoms could be replaced by a number of other divalent and trivalent ions such as Mg, Cu, and Co; that is, as impurities. Such a mineral is intensely absorbing because of the resonance between Fe^{++} and Fe^{+++} atoms,

* The perfect unit cell of a spinel contains 32 O ions and 24 sites for occupation by divalent and trivalent metal ions. Each divalent metal ion coordinates tetrahedrally with 4 oxygen ions; each trivalent metal ion coordinates octahedrally with 6 oxygen ions; and each oxygen ion binds 1 divalent and 3 trivalent metal ions.

and its electron conductivity is somewhat less than that of a semiconductor, but it is suggestive that electron conduction may occur readily enough. In the structure of spinel the exact distribution of cations is of secondary importance, so there may be "defect structures"; that is, regions of excess positive or negative charge, and even the absence of metal ions, as in the γ iron oxides. Materials of this kind might resemble transistors, and their properties would provide a system for the storage and transfer of electrons detached by the light. Contamination with sulfur might provide a rectification layer through which electrons might pass in only one direction.

Thus, one might imagine a defect crystal structure, perhaps of only several hundred atoms in extent, in which electrons of the mineral were freed and caused to move only one way, from the hydroxyl toward the sulphur atom on exposure to light (FIGURE 4). The mineral could trap several photons and conduction would permit a cooperative reaction to occur. The result would be a production of reductant on the upper surface and of oxidant on the lower surface. MnO_2 could interact with the peroxide intermediate to form O_2.

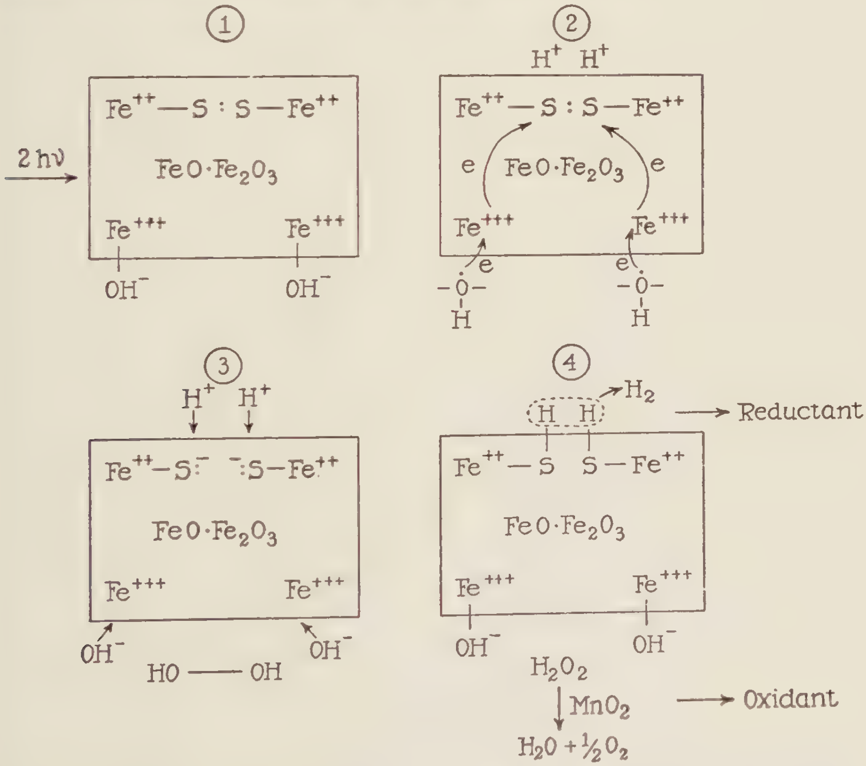

FIGURE 4. Diagram of speculation on the structure of a mineral photocatalyst that would decompose water to H_2 and O_2. The diagram shows a spinel structure of a magnetite that is contaminated with sulfur atoms at the upper surface. Two photons of visible light act to cause two electrons to flow from the OH groups on the lower surface toward the S—S group, which accepts the electrons and is reduced to two SH groups. The two oxidized OH radicals form an intermediate peroxide.

With a mechanism that might bring about photodecomposition of water, we would be provided with a source of chemical energy in terms of oxidant and reductant. With this chemical energy, carbon and nitrogen compounds could be converted to complex organic molecules. The catalysts for these conversions could be metal ions that might reside in the energy unit as impurities.

Evolution of the Chloroplast

The photosynthetic organ of higher plants is the multigranum chloroplast. Within the semipermeable membrane of the chloroplast, embedded in a colorless stroma, lie cylindrical green grana. In spinach there may be about 40 grana per chloroplast, each of about 5000 Å. diameter. The grana are composed of stacks of flattened disks, from 20 to 100 in number, depending on the species. The disk is a wafer-shaped structure that consists of a membrane 35 to 40 Å. thick (that is, of the diameter of a protein molecule) that encloses a space some 65 Å. in height. There is enough chlorophyll in the granum so that, if one assumes the chlorophyll to be localized in the disk, this would suffice for the formation of a monolayer of chlorophyll molecules in the disk. The stroma also contains delicate membranes that run from one granum to another.[29]

On the basis of phylogeny one may trace a progressive development of the chloroplast, beginning with the photosynthetic bacteria. In the photosynthetic purple bacterium *Rhodospirillum rubrum*, grana of about 1100 Å. in diameter that contain bacteriochlorophyll, proteins, and a small amount of pentose nucleic acid have been reported. From the blue-green alga *Synechococcus cedorum* grana of about 2200 Å. in diameter have been obtained. Chlorophylls and carotenoids were associated with the grana, but phycocyanin was lost into solution. In neither the grana of bacteria nor those of blue-green algae have layered structures been reported. Perhaps these grana represent single coarse disks. The structure and organization of the grana must be considered as highly advanced, even in these "primitive" organisms.

In many algae the chloroplasts consist of a single granum composed of a pile of dense parallel lamellae, perhaps interpretable as being the membranes of the disks. For example, *Euglena* possesses about 21 dense lamellae, and each of them is composed of a double membrane that is separated by less dense material. The spiral chloroplast of *Spirogyra* consists of a series of parallel extended lamellae stacked one upon another. These chloroplasts, consequently, are single-granum chloroplasts in contrast to the multigranum chloroplasts of higher plants.

In some green algae such as *Chlamydomonas* and in chloroplasts of higher plants, transition stages may be seen from single grana to multiple grana. The multiple grana are more or less widely separated, though seemingly connected by the delicate parallel membranes of stroma material.

Most of the evidence supports the idea that a chloroplast is a self-perpetuating body which, if lost from the cell, is never regained. Some evidence suggests that nucleic acids are present in these bodies. The chloroplast of lower plants is seen to multiply by fission. In plants where growth occurs by way of meristematic cells the chloroplast appears to be carried along in the form of a tiny primary unit of the plastid, the "proplastid." This body, 5000 Å. in diameter,

contains a submicroscopic granule of nucleoprotein, the elementary granule of Strugger.[30] Thus, at the growing points where the cell specializes in the rapid formation of nucleic acids and nuclei toward the manufacture of new cells, the development of the chloroplast is inhibited, although fission of the proplastids occurs. Behind the meristematic cell the new cells enlarge, protein synthesis becomes dominant, and the proplastids develop into chloroplasts. A transition stage in chloroplast reduction during rapid cell multiplication may be represented by the condition found in the fern sporophyte of *Isoetes*. Here, the meristematic cells of the root and the leaf are found to contain a single chloroplast that elongates and divides prior to cell division; the daughter plastids pass to the opposite poles of the spindle. As the cells mature they come to contain a variable number of plastids.

Summary

Speculations are made on the origin of preprotoplasm; these are based on the assumption that the conditions on the primitive earth were not necessarily far different from those obtaining today. Water, sunlight, minerals, and an anaerobic atmosphere are assumed. The hypothesis is proposed that one may trace the primary mechanisms for the functioning of protoplasm back to inorganic origins.

An impure magnetite is proposed as the basic elementary energy unit that might perform the photodecomposition of water with visible light. Oxidants and reductants thus would be generated to supply the chemical energy for the conversion of carbon and nitrogen compounds into complex organic materials in the neighborhood of the elementary unit. Metallic ions, present as constituents of the minerals, would serve to catalyze the same reactions that they now catalyze as metalloenzymes.

The catalysts of respiration and photosynthesis (namely, heme and chlorophyll) may have developed from simple precursor organic compounds which, as metal complexes, gradually took over the functions of the elementary energy unit. The biosynthetic chain of the porphyrins continued to develop so that the end products would be more efficient catalysts. Eventually, the two functions of photodecomposition of water and oxidation with formation of water came to reside in two separate cytoplasmic units, the chloroplast and the mitochondrion.

Phylogenetically, the chloroplast may be traced from a single granum without lamellae, to single-granum chloroplasts with dense lamellae, to chloroplasts with many grana and fine lamellae. The chloroplasts are self-duplicating bodies that appear to contain nucleic acid. Their properties in connection with photosynthesis are discussed.

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PHOSPHORUS AND THE ORIGIN OF LIFE

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I cannot resist inflicting a personal item upon the reader. The conference upon which this monograph is based marked a centennial occasion in my family, and the place and circumstances accented the fact. In 1856 my father, John Thomas Gulick, through the Lyceum of Natural History in the City of New York, as The New York Academy of Sciences was then called, published the first paper[10] in his lifelong study of evolution under isolation as illustrated by the land snails of the Hawaiian Islands.[8, 11] His studies were inspired, I might add, by *Zoology of the Voyage of the Beagle*, Darwin's account of his trip to the Galapagos Islands for, of course, *On the Origin of Species by Means of Natural Selection* had not yet been published.

Our object in this monograph is to contemplate the earliest beginnings of life. This question of how life originated basically is an inquiry into the origin of the distinctively biological mode of action.[12, 18, 22] Anything that can be considered to be *alive* must handle chemical energy in a regulative manner: it must capture material and energy from its surroundings, it must undergo growth and reproduction and, it would seem, it must achieve all of this primarily through the agency of organic catalysts (enzymes) which it shapes for itself. Anything less would fall short of being the starting point for life.

The organism's fundamental need for the element phosphorus seems to be based largely on these dynamic reasons. Among the elements found in the protoplasm there are three that serve distinctively in the regulated transfer of chemical energy; namely, sulfur, iron, and phosphorus.[3] Of these, phosphorus performs this function with much the greatest facility, because of its ability to activate one of its contained electrons with a unit of energy that later can be employed to complete a chemical reaction. Without such minutely divided stores of power it would be quite impossible for biological catalysts to condense small organic molecules by a series of small endothermic reactions into one long, chain-shaped giant molecule.

Many investigators believe that an essential factor in all polypeptide synthesis is nucleic acid, a substance that is itself a chain molecule, but is formed of phosphoric esters. This appraisal of the function of nucleic acid appears to me to be probably a valid one. It has been noted elsewhere in these pages that the internal spacing of this molecule agrees reasonably with the peptide spacings in proteins, and we can interpret this as an advantageous distribution of its energy-lending electrons. If we assume that the mechanics of self-reduplication have conformed to the same basic laws ever since the inception of life, then we must postulate that at least something comparable to the giant chain-shaped molecules of nucleic acid was on hand from the very beginning.

This requirement brings us to a dilemma.[7, 9] Imagine such phosphate chains taking form through random meetings in a sea water that contained the mineral

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content of the present oceans; that is, in the range of 0.005 to 0.040 parts per million (ppm) or even in a saturated solution of phosphate in sea water, which is roughly 0.125 ppm of the element! Concentrations in excess of that will precipitate and gradually will build concretions of the mineral apatite. The supply of P today is always good in living protoplasm and in cell sap; there are 250 to 750 ppm totals in herbaceous leaves and 290 to 610 ppm in the seaweed *Ulva*.[13] Of the latter, about 20 to 40 ppm are recorded as inorganic phosphate P within 15 min. of tissue maceration. This compares with the 30 to 60 ppm of human blood plasma.

The problem of the solubility of phosphorus that is posed here may be resolved, I believe, by a reference to oxidation potentials. We are all familiar with the argument that our atmosphere contained no free oxygen[12, 18, 21, 24] until plants introduced it as a by-product of photosynthesis. A. C. Lane, the geologist (1917), argued that the first appreciable free oxygen appeared at about the time of the Laurentian Revolution in the Precambrian epoch.[15] The evidence that this author cited was the degree of oxidation of the iron in the deposits before and after this unconformity. Recent estimates date this revolution as having occurred about 1450 million years ago.*

How will the solubility of P be affected by such a difference in the level of oxidation? All natural P on the earth's surface today is in the form of phosphate or its equivalent; that is, it is fully oxidized. Phosphate, if it is in acid solution, is all but totally precipitated in the presence of either Fe or Al, which are scarcely ever absent simultaneously. The ubiquitous Ca ion is a highly effective precipitant in neutral or alkaline media. Consequently it is no wonder that, in spite of its widespread fairly abundant occurrence in magmatic rocks, the scarcity of *assimilable* P is often a limiting factor in today's environments. However, if we turn to levels of oxidation lower than those now current, calcium phosphite begins to be somewhat soluble and, still further down the scale, calcium hypophosphite is 500,000 times as soluble as is the phosphate; indeed, it is capable of supplying any biologically desirable concentration. From these data comes what may be termed the "hypophosphite hypothesis" of the origin of life.

Our next problem is to attempt to trace the implications of this hypothesis and to picture what the world would be like if the general redox level were such as to permit a widespread occurrence of aqueous hypophosphite solutions. Free oxygen would be entirely out of the question. The chief combined forms of oxygen would be water, silica, alumina, and the oxides of alkaline and earthy metals. Sulfates could not exist, but only sulfides and sulfhydryl compounds. All metals with variable valences, including iron, copper, and tin, of necessity would be reduced beyond the degree that would permit their behavior as electron acceptors, since otherwise all phosphorus would soon be fully oxidized into phosphate, probably at the expense of oxygen catalytically released from water. Water itself would have to remain sufficiently cool to

* W. W. Rubey has called to my attention the fact that recent datings by means of radioisotopes have revealed extensive deposits of ferric oxide very much older than the Laurentian Revolution. Consequently, it seems that we are only at the beginning of the problem of the relative chronology of ferrous and ferric sedimentary rocks.

resist the decomposition by which it would yield its oxygen to phosphorus. None of the familiar oxygen-hydrogen donor-receptor mechanisms (except phosphorus anions) could be functional, although ultraviolet light could be expected to take their place to a considerable degree. Nitrogen would reveal no affinity for oxygen, but it would have a very pronounced affinity for hydrogen and carbon. Carbon would be found chiefly in combination with nitrogen and hydrogen, some of the latter being desaturated photochemically. CO could exist, at least temporarily, since it would be generated through the thermal reaction, $C + H_2O \rightarrow CO + H_2$. This, it seems, would not be a poison as long as the cytochromes did not exist. If warmed or catalyzed, it would be liable to rearrangement – $2\,CO \rightarrow C + CO_2$ and this would be followed by a tendency of the CO_2 to lose oxygen to any good receptor. Large-scale CO_2 would not be possible.

Unoxidized phosphorus is known to mineralogists only as the phosphides that have been observed in meteorites. The typical composition in this case seems to be Fe_2NiP. It is logical to infer that comparable minerals are native to the deeper layers of the earth and that, formerly, they were not excluded from surface rocks. With moisture and very slight oxidation such phosphides at first should yield hypophosphite; next, with more oxidation, they should produce the other acids of phosphorus.

The carbon-nitrogen precursors with which the organism at first sustained itself must have been largely devoid of oxygen. This element must have come mainly from water and, probably, also from carbon monoxide. The earliest amino acid syntheses consequently would have been along lines rather different from those of the present,[1, 2, 14, 17, 18] or from those that are set up under most other hypotheses of spontaneous generation. I do not believe that the difficulties of such synthesis would be any greater than under aerobic conditions.

The question of biological energy in the times before plant photosynthesis began also may call for a revised estimate. Photochemical processes seem more likely to have built up a supply of unsaturated hydrocarbons, nitriles, and the like than to have generated much fermentable substrate. It would seem most likely, then, that the chemical energy was derived from the hydration of double and triple bonds or, conceivably, from such a reaction as the breakdown of carbon monoxide ($2\,CO \rightarrow C + CO_2$) and not from fermentations until some later date. Decarboxylations might also have occurred quite early.

When eventually photosynthesis did begin, did it utilize CO_2 or CO?[4, 19, 20, 25] This is not an idle question, because it is known that CO does not always poison the chlorophyll mechanism, and that some plants can build carbohydrates from it efficiently. The solution to this problem is not easy, as there are processes that generate both gases, as well as processes that dispose of both; carbon monoxide becomes graphite and carbon dioxide, and dioxide, in turn, disappears as mineral carbonate.

It may be asked whether some redox facilitator other than phosphorus may not have played the earliest role. We return at once to the two other active elements, sulfur[5, 6, 16, 23] and iron. Both of these appear to be less probable agents than does phosphorus, because we do not find in nature any actual pattern by which either of them would deliver small units of chemical energy at

regularly spaced intervals along the filament of a giant molecule. In the case of iron, I do not see how one could set up such a mechanism. Sulfhydryls crowded at close intervals all along a protein filament could be pictured on paper, but we have no precedent for such a thing in nature. The implications in such an alternative hypothesis would seem to be these: the redox level would have to stand within the range in which the reaction 2—SH → —S—S— + 2H would be actively reversible, since beyond that range it could not mediate the transfer of chemical energy. This means a voltage slightly negative by comparison with that of the standard hydrogen electrode. All phosphorus would be fully oxidized, as it is today, and both methane and carbon dioxide would be stable compounds, more stable, indeed, than carbon monoxide. All cytochromes would stay reduced, thus preventing them from having any redox catalytic influence. Free oxygen could not exist in more than traces, because more than that would convert all sulfhydryls irreversibly into disulfides and thus nullify their power to transfer energy by way of hydrogen atoms. Consequently, what I should describe as the one imaginable (but scarcely probable) alternative to the hypothesis of hypophosphites (or phosphites) agrees with the latter in demanding a world bereft of free oxygen. In several other aspects, however, we have found that the two suppositions picture rather different worlds.

In conclusion, to sum up our tentative findings as based on the hypophosphite hypothesis, we may imagine an atmosphere and a hydrosphere enriched with methane and other hydrocarbons (some of them unsaturated) and with ammonia, cyanogen, or cyanide and, probably, carbon monoxide. Further, there would be numerous more complex, nonvolatile photochemical products in the hydrosphere. The list of essential ingredients would be completed by the action of water corroding away the phosphides out of the pre-Precambrian rocks. Thus the dynamic needs, as well as the ponderable materials, became available.

It will be seen that in this paper I have taken sides sharply on more than one very controversial topic. In this connection it should be remembered that these problems involve exceedingly protracted periods of time. So it is possible that seemingly contradictory conditions actually may have occurred, but at different points in the process.

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THE INTERACTION OF SYNTHETIC POLYNUCLEOTIDES*

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The enzyme polynucleotide phosphorylase was discovered in extracts of *Azotobacter vinelandii* by Grunberg-Manago and Ochoa.[1, 2] The enzyme catalyzes the reaction

$$nX\text{—}R\text{—}P\text{—}P \rightleftharpoons (X\text{—}R\text{—}P)_n + nP \qquad (1)$$

where R stands for ribose, P for phosphate, and X for adenine, guanine, inosine, uracil, or cytosine. Magnesium ion is required. The product $(X—R—P)_n$ is a polynucleotide of high molecular weight. Some recent work by Ochoa[3] and his collaborators will be summarized before consideration of the physical properties of the polymers. This work on the structure of the polynucleotide has shown that it is a linear polymer of nucleotides connected by phosphodiester bonds between the 3′ and 5′ hydroxyls of adjacent nucleotides. The polynucleotide is thus structurally equivalent to ribonucleic acid (RNA). The reaction can be described as a polymerization that results from the splitting of orthophosphate between a 5′ nucleoside diphosphate and the 3′ hydroxyl on the ribose moiety of another nucleotide. The reaction is reversible and, in the reverse direction, it results in the phosphorolysis of the polymer by addition of orthophosphate across the diester bond. Equilibrium is attained when 60 to 80 per cent of the phosphate available according to EQUATION 1 is in the form of orthophosphate. The enzyme has been named polynucleotide phosphorylase by analogy with the action of phosphorylase on glycogen.

If the reaction is performed with a single kind of nucleoside diphosphate, a homopolymer that contains only one type of base is obtained. If the enzyme is presented with a mixture of diphosphates, a copolymer is synthesized. The existence of internucleotide bonds in the copolymers between nucleotides that contain different bases has been proved by Heppel[4] and his group by detailed examination of the chemical and enzymatic degradation products of the polymers. While a large variety of copolymers can be made, the one that has received the most attention is that containing adenylic, uridylic, guanylic, and cytidylic acids, the four nucleotides present in RNA (poly AGUC). The composition of the polymer with respect to the molar ratios of the bases depends somewhat on the relative concentrations of the respective diphosphates in the reaction mixture. However, when equimolar concentrations of the diphosphates are used, a polymer is made that contains the bases in about the same proportion as in *Azobacter* RNA, although this proportion is a considerable departure from equimolarity. Other properties that indicate the similarity of poly AGUC to RNA are the molecular weight,[5] the nature of the end groups on the nucleotide chains,[6] the X-ray diffraction pattern, and the rate of phosphorolysis.[6] It is apparent that the product is indeed similar to RNA.

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Further work with the enzyme and its role in the bacterial cell certainly will contribute to our understanding of the biosynthesis and function of RNA. Another way in which these developments have been of importance results from the unique structure of some of the polymers and the ways in which the study of their properties relates to the structure and properties of nucleic acids. The work described here is concerned with the latter approach. The particular advantage offered by the polymers is that they can be prepared as homopolymers with a single kind of nucleotide unit instead of with four different nucleotides, as in RNA. The simplification in interpretation resulting from the presence of a single monomer unit may permit deductions that could not be drawn from experiments on the more complex RNAs.

The most interesting result of the investigation of the physical properties of the polymers has been the finding that polyadenylic acid (poly A) and polyuridylic acid (poly U) interact simply on mixing them together at room temperature. These form a stable aggregate of the two that has a greatly increased molecular weight. This property was first noted in some experiments that were performed to determine whether the copolymer of adenylic and uridylic acids (poly AU) was in fact a copolymer or whether it was a mixture of poly A and poly U. Such conditions for electrophoresis of the polymers were found that a substantial difference in mobility was established between poly A and poly U. At *p*H 9.6 at 0° C. and ionic strength 0.1, poly A had a mobility of 10.6×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ and poly U, 13.2×10^{-5}. This is sufficient to permit resolution of the two in a mixture. However, when a mixture of the two was run in the same buffer, a single peak that had a mobility of 11.1×10^{-5} was obtained. The copolymer also gave a single boundary with a mobility of 12.7×10^{-5}. These experiments do not give an answer to the original question. This was solved subsequently by Heppel and his group[4] by analysis of the products of hydrolysis. However, the electrophoresis experiment on the mixture shows that the two components do not migrate independently; instead, they form a single species with intermediate properties.

A similar experiment performed in the ultracentrifuge gives a clearer picture of the reaction. The sedimentation coefficient of one sample of poly A in a phosphate buffer at *p*H 7 was 2.5 *S**. Under the same conditions poly U showed 2.2 *S*. When the mixture of the two was sedimented, a coefficient of 5.3 *S* was obtained; no trace of slower-sedimenting material was present. The polymers used in this experiment had number average molecular weights of about 80,000, as determined by end-group analysis.[5] The results of another experiment in which diffusion coefficients also were measured in order to determine the molecular weights of the polymers is shown in TABLE 1. Different samples of polymer were used here, and the poly U had a very low molecular weight. All of the polymers are heterogeneous with respect to molecular weight; average values are given in the table. The mixing is seen to yield a species with an average molecular weight more than twice that of the poly A and twenty times that of the poly U.

Additional evidence regarding the poly A-poly U interaction comes from the

* *S* = Svedberg unit = 10^{-13} seconds.

TABLE 1

MOLECULAR WEIGHT OF POLY A, POLY U, AND THEIR AGGREGATE

	$D^{A}_{20,w}$ cm.^{2} sec.$^{-1}$ $\times$ 10^{-7}	$S_{20,w}$ Svedberg units	Molecular weight
Poly A	1.7	5.3	177,000
Poly U	7.4	2.2	17,300
Poly A plus poly U	1.3	9.9	450,000

D^{A} is the height-area average diffusion constant determined in a Gouy diffusiometer at a polymer concentration near 0.2 per cent.

The sedimentation coefficient was obtained by extrapolating to zero the results of three runs in the Spinco ultracentrifuge at concentrations of 0.6, 0.3, and 0.15 per cent.

The molecular weight was calculated assuming the partial specific volume to be 0.58.

increase in viscosity and the changes in ultraviolet spectra that occur on mixing. The latter have been investigated in some detail[9] but, before describing them, the spectra of the homopolymers must be considered. The spectrum of each polymer was obtained in phosphate buffer at pH 7 and was compared with the equivalent concentration of mononucleotide. The latter curve was obtained by hydrolyzing the polymer in 1 M NaOH at room temperature for 18 hours. The spectra also were measured in water, 6.4 M urea, and 0.1 M HCl. In the cases of poly A, polyinosinic acid (poly I) and polycytidylic acid (poly C), the spectrum of the polymer falls below that of the constituent mononucleotides by 20 to 40 per cent at the wave length of maximum absorption, and this maximum is shifted to lower wave lengths by 2 to 3 mμ. The polymers thus show the "hyperchromic effect" that is well established both for DNA and RNA.[10] This effect has been thus named because of the increase in absorption that accompanies the hydrolysis of the nucleic acid to mononucleotides. As compared with the spectrum in phosphate buffer, in the absence of salt the spectrum showed increased extinction for poly I and poly C and decreased extinction for poly A. The addition of urea to 6.4 M increased the extinction in each case, although not to the level of the mononucleotide.

In contrast to these changes the extinction of poly U is only slightly below that of the constituent mononucleotides, and the spectrum was the same at various ionic strengths and concentrations of urea. The spectrum of polyguanylic acid (poly G) has not been investigated extensively because only small yields of the polymer can be obtained, and the nature and molecular weight of the product are uncertain. The liberation of phosphate is very slow during the synthesis of poly G, and it ceases when about 10 per cent has been liberated. Most of the product corresponding to this phosphate liberation is lost on dialysis of the reaction mixture.[3]

When poly A and poly U are mixed, the observed extinction is not only lower than that of the constituent mononucleotides, but also about 30 per cent below the value expected for additivity of the polymer extinctions. The additional drop in extinction is a consequence of the aggregation reaction that was directly demonstrated by electrophoresis and sedimentation. The change in ultraviolet spectrum accompanying mixing provides a convenient means of

determining some of the characteristics of the reaction. Some observations made in this way are:

(1) The extinction of the aggregate is increased in 6.4 M urea solution.

(2) When the temperature of a solution of the aggregate in phosphate buffer is increased a sharp rise in optical density occurs just before a temperature of 60°C. is reached. The optical density falls again to its previous value on cooling.

(3) In the absence of salt the extinction is that expected on the basis of additivity of the components, which indicates that the aggregate is not formed. The addition of $MgSO_4$ to a concentration of 10^{-4} M or phosphate buffer to an ionic strength of 10^{-2} results in an immediate fall in optical density. The density is not further decreased by raising the magnesium ion or salt concentration above these levels.*

(4) No other pair of polymers than poly A and poly U interact under the conditions that we have used. It should be noted, however, that the experiments with poly G may not be definitive because of the uncertainties mentioned above as to the nature of the substance.

The aggregation of poly A and poly U is explained most directly by assuming that adjacent polynucleotide chains are held together by the formation of hydrogen bonds between adenine and uracil. The existence of hydrogen bonds is supported by the changes in spectrum in concentrated urea and by an increase in the temperature. The fact that the ultraviolet spectrum is modified under these conditions indicates that the bases are probably directly involved. The salt and magnesium ion effects are consequences of the large electrostatic barrier to the association of similarly charged chain molecules. Bonding forces are sufficient to cause aggregation when this barrier is reduced by the binding of magnesium or by increasing the ionic strength of the hydrogen.

Similar considerations indicate that the spectral changes in the homopolymers are a result of hydrogen bonding between two molecules of the same purine or pyrimidine either on an intermolecular or intramolecular basis. The number average molecular weight of poly A and of poly U as determined by end-group analysis is of the same order as that estimated from sedimentation experiments, whereas for poly AU and poly AGUC the sedimentation molecular weight is tenfold that obtained by end-group analysis. The copolymers are therefore highly associated as compared with the homopolymers. A more complete analysis of the molecular weight distributions will be required in order to see whether a clear distinction between intramolecular and intermolecular interaction in these two classes of polymers can be made. The behavior of the copolymers in this respect indicates that RNA is also highly associated.

It has been widely assumed that hydrogen bonding of the type discussed above is of importance in maintaining the specific structure of deoxyribonucleic acid (DNA). Pairs of hydrogen bonds between adenine and thymine and between guanine and cytosine are central features of the Watson and Crick doubly stranded model of DNA.[11] The adenine-uracil interaction on which the

* This result was obtained by J. D. Smith of Cambridge University, England. Our early samples of polymer apparently were contaminated with traces of magnesium salt, and they interacted in water.

poly A-poly U aggregation is based corresponds to the first pair of Watson and Crick. The information from the spectra of the polymers indicates that there is a specificity in the formation of hydrogen bonds among the bases. According to the survey of hydrogen bonding possibilities by Donohue,[12] pairs of bonds can be formed between any two bases of the same kind or any of the various combinations of two different bases. The ultraviolet absorption of the homopolymers shows that each, except poly U, forms such bonds under our conditions. However, of the possibilities among different bases only the interaction of poly A and poly U could be demonstrated. It is, of course, possible that some of the pairings that can be formed are not favored because of competition between the breaking of intramolecular bonds and the reformation of bonds intermolecularly. The behavior of the poly A-poly U mixture shows that the hydrogen-bonded structure can be formed spontaneously, that it is sufficiently strong to overcome a considerable electrostatic barrier to its formation, and that it is not dissociated by dilution to the concentrations of 0.001 per cent that are used in studies that utilize ultraviolet radiation. This structure also demonstrates directly the correlation between hydrogen bonding and the hyperchromic effect. This correlation has been assumed to hold for DNA, and the changes caused by ultraviolet radiation under various conditions have been used by several investigators to indicate the stability of the hydrogen-bonded structure.[13, 14]

When DNA is treated in a way that would be expected to break the hydrogen bonds, such as exposure to high concentrations of urea, heat, or extremes of *p*H, the extinction increases irreversibly. Urea and heat bring about the same change in the homopolymers as in the poly A-poly U aggregate. In contrast to DNA, the polymers change reversibly, and the original extinction is recovered on cooling or on reducing the concentration of urea by dilution or dialysis. These differences may result from the simplicity of arrangement of the poly A-poly U aggregate which should permit pairing in every adjacent position of the two chains. In DNA only certain specific adjacent positions will permit pairing because of the probable variation in base sequence along any single chain and the necessity of matching this with a unique complementary sequence in the adjacent chain.

It should not be presumed that the aggregate of poly A and poly U necessarily has the same structure as DNA because of these similarities in behavior. The similarities, however, may extend to certain aspects of the arrangement of the chains in helices. Fibers prepared from several of the polymers have been examined by X-ray diffraction by Alexander Rich.[7] That investigator has found that the patterns obtained from poly A indicate a more highly oriented structure than do RNA, poly U, poly AU, or poly AGUC. A highly oriented fiber pattern was obtained from the mixture of poly A and poly U, which Rich and Davies[15] have interpreted as due to a doubly stranded helical structure. Although the dimensions of the helix differ slightly from those of DNA, the same complementary pairing of bases attached by hydrogen bonds is believed to stabilize the structure.

Another consequence of the interaction of poly A and poly U has been found by Ochoa[6] in the reduction in the rate of phosphorolysis of the mixture. Poly A

and poly U alone are readily phosphorolyzed by the enzyme. The rate of this reaction can be followed in the presence of orthophosphate P^{32} by the appearance of the isotope as organic phosphate. The mixture was phosphorolyzed at about 20 per cent of the rate of the separate polymers. Poly AGUC and several preparations of RNA also were phosphorolyzed slowly. The only exception among the RNAs was that of tobacco mosaic virus, which was phosphorolyzed at a rate of about half that of poly A. The formation of aggregates thus inhibits phosphorolysis, perhaps by making the sensitive bond less available to the enzyme. This effect should place a limitation on the reversibility of the over-all reaction when copolymers are being synthesized, and it may be an important effect in the biosynthesis of RNA. The comparative rates of phosphorolysis also provide evidence for association of nucleotide chains in most RNAs.

One facet of the general considerations of the spontaneous generation of life is the spontaneous generation of order at the molecular level. The formation and stability of the highly ordered structures of biological macromolecules usually have been explained by the operation of the secondary valence forces of electrostatic interaction, hydrogen bonding, and van der Waals' interaction. Of these, hydrogen bonding has been particularly attractive because of the specificity that can be obtained by fitting together complementary patterns of donor and acceptor atoms. The forces are weak, and they must be multiplied by the formation of many bonds over the extent of a large structure to be effective stabilizing forces. In spite of the firmly established role of hydrogen bonding, there are few cases in which the ordered structure is formed spontaneously either on adding together two species with complementary properties with respect to hydrogen bonding or on changing the environment to permit the formation of intramolecular bonds in a single species. An example that comes to mind is the reversible formation and dissociation of the α-helix in synthetic polypeptides. This has been observed by Elliot[16] on heating and cooling, and by Doty and Yang[17] on changing the solvent. The reconstitution of the tobacco mosaic virus from the separated RNA and protein by Fraenkel-Conrat and Williams[18] probably falls in this category also. The aggregation of the synthetic polynucleotides provides another such example; it is one that is probably more versatile than the others from the standpoint of experimental investigation because of the availability of several different kinds of polymers of varying molecular size, the apparent specificity with respect to the required base composition, and the possibilities for intramolecular as well as intermolecular interaction.

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THE STRUCTURE OF CRYSTALLINE PROTEINS

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There are two main classes of proteins: the fibrous and the globular. A fibrous protein consists of long molecules of somewhat variable molecular weight that are arranged more or less parallel to each other along the direction of the fiber axis. The X-ray diffraction patterns of these substances sometimes indicate considerable regularity in their molecular patterns, but even the most highly ordered of these are far from being the beautiful periodic arrays characteristic of the internal structures of true crystals. In general, fibrous proteins are soluble only through decomposition by the chemical action of a solvent.

A globular protein, on the other hand, usually consists of molecules of the same molecular weight and, apparently, of the same size and shape. Mixtures of such proteins are usually found in solution in living matter and can often be crystallized out. In some cases it has been possible to separate from these mixtures protein fractions that form crystals of great perfection, comparable in quality to minerals. These crystals produce X-ray diffraction patterns that indicate almost perfect order of molecular arrangement; each molecule is surrounded by other molecules in exactly the same way. It is with crystalline proteins such as these that I propose to deal in the following paragraphs.

About 200 globular proteins have been crystallized, and about 50 of these have been measured by X-ray diffraction methods. These measurements give data from which the sizes, shapes, and symmetries of the unit cells can be computed. The unit cell of a crystal is a parallelepiped (the three-dimensional analogue of a parallelogram) which contains the smallest structural unit from which the crystal can be built up by repetition without rotation. Except for triclinic crystals with no symmetry, the unit cell contains more than one asymmetric object. Therefore, since most protein molecules are without symmetry and most protein crystals have rotational axes of symmetry, there is almost always more than one molecule in the unit cell of a protein crystal, the minimum number of molecules being determined by the particular combination of rotation axes present. The number of protein molecules in a unit cell is thus some multiple of a number that is characteristic of the symmetry of the crystal—in most cases this multiple turns out to be unity.

An estimate of the molecular weight of a globular protein is usually available from physicochemical data. This figure, together with the density and solvent content of the crystal, can be used to calculate the approximate number of protein molecules in one unit cell; a comparison of this number with that required by symmetry makes possible an exact evaluation of the number of molecules per unit cell and, therefore, of the molecular weight of the protein. Table 1 sets forth the results that have been obtained in this way.

The shape, size, and symmetry of the unit cell of a crystal put definite restrictions on the size and shape of the asymmetric units of which the crystal is

TABLE 1

Protein	Solvent	Space group	Molecules per unit cell	Lattice constants (Å.) a_0	b_0	c_0	β	Volume of cell (Å.^{3})	Density	Per cent solvent	Molecular weight
α Chymotrypsinogen D	H_2O	$P2_12_12_1$	4	42.6	54.6	91.9	—	214,000	1.215	35.8	25,000
Edestin	H_2O	F (cubic)	4	122			—	1,816,000	1.306	0	360,000
Excelsin	H_2O	R32	3	82.8		196.2	—	1,164,000	1.298	0	303,000
Ferritin	H_2O	$P2_12_12_1$	4	131.5	131.5	186	—	3,216,000	1.45	35	545,000
Apoferritin	H_2O	F (cubic)	8	186				6,455,000	1.27	33	460,000
Horse methemoglobin	H_2O	C2	2	109	63.2	54.4	111°	349,000	1.160	45.1	67,000
Horse methemoglobin	25% Alcohol	C2	2	102	51.4	47	130°	189,000	1.270	7.4	66,700
Insulin zinc complex	Acetone + phosphate buffer	R3	18	74.8		30.9		150,000	1.312	5.35	6,250
β Lactoglobulin I	H_2O	$P2_12_12_1$	8	69.29	70.42	156.47		763,500	1.144	46.2	35,400
β Lactoglobulin I	H_2O	$P2_12_12_1$	8	60.7	61.0	112.4		416,200	1.259	9.8	35,600
β Lactoglobulin I	H_2O	$P2_12_12_1$	8	60.1	60.5	111.5		405,000	1.257	11.2	34,000
β_2-Lactoglobulin III	H_2O	$P2_1$	2	36.1	127.5	36.0	106°05′	164,000	1.162	35.5	35,800
Chick lysozyme chloride	H_2O	$P4_12_1$	8	71.2		31.4		159,000	1.305	9 H_2O 2.45HCl	13,900
Ribonuclease II	Aqueous alcohol	$P2_1$	2	29.10	30.08	51.03	114.0	40,800	1.269	13.6	13,400
Serum albumin	H_2O decanol	F2	8	178	51	166	91°	1,596,000	1.145		65,200
Serum mercaptalbumin	7% Alcohol in H_2O	$P2_12_12$	2	165	83	63	—	863,000	1.135	55.4	131,500
Tobacco seed globulin	H_2O	Trigonal	3	88		201		1,347,000	1.293	0	350,000
Tobacco seed globulin	H_2O	F (cubic)	4	123	—	—		1,861,000	1.287	10.4	325,000
Tobacco necrosis virus	$(NH_4)_2SO_4$ solu.	P1	2	157	154	147	100° = α, 110° = β, 120° = γ	2,572,000	1.317	21.4	1,600,000
Turnip yellow virus	$(NH_4)_2SO_4$ solu.	Cubic	8	528	—		—	147,000,000	1.45	0	2,800,000

TABLE 2
CRYSTALLINE MODIFICATIONS OF RIBONUCLEASE

Modification	Solvent	Space group	a_0 (Å.)	b_0 (Å.)	c_0 (Å.)	β	Z	Cell volume (Å.^{3})
I (Ni_3 complex)	55% 2-methyl-2,4-pentanediol, pH = 6.60	$P2_12_12_1$	44.48	75.74	37.71	—	4	127,000
II	50% tert-butyl alcohol, pH = 5.0	$P2_1$	30.28	38.39	53.16	105.83°	2	59,450
III	50% n-propyl alcohol, pH = 5.0	$P2_1$	42.91	45.38	77.2	114.31°	4	137,000
IV	67% n-propyl alcohol, pH = 5.0	$P6_222$	88.3	—	112.6	—	24	761,000
V	70% 1,3 propanediol, pH = 7.0	$C222_1$	31.60	61.98	121.8	—	8	239,000
VI* (dye complex)	50% tert butyl alcohol, pH = 5.2	C2	70.60	38.99	51.65	103.96°	4	137,980
VII (Ni complex)	55% 2-methyl-2,4-pentanediol, pH = 7.05	$P2_1$	46	28	46	102°	2	58,000
VIII (Cu complex)	55% 2-methyl-2,4-pentanediol, pH = 5.57	C2	58.65	53.92	43.40	119.65°	4	119,300
IX	55% methyl alcohol, pH = 5	$P2_12_12_1$	42	53	46	—	4	102,000

* The dye is tetraiodophenolsulfonphthalein.

built. If, as is often the case with crystalline proteins, the asymmetric unit consists of one molecule and its associated solvent, the molecule itself is also subject to these restrictions. For instance, the distance across a molecule in the direction of a row of lattice points in the crystal cannot be greater than the distance between these lattice points. Thus, if a particular protein crystallizes in a number of different modifications, a reasonably accurate idea of its molecular shape can be inferred. This has been done for hemoglobin by the group at Cambridge University, Cambridge, England, with the result that the hemoglobin molecule is known to be roughtly ellipsoidal in shape, with principal diameters approximately 55 Å., 55 A., and 65 Å.

The enzyme ribonuclease is a protein that can be prepared in at least nine different crystalline modifications. Their unit cells are listed in TABLE 2, based on measurements made in the laboratory of the Protein Structure Project of the Polytechnic Institute of Brooklyn. It will be noted that in each of modifications II, V, and VII one of the unit cell dimensions is close to 30 Å. This suggests strongly that one of the molecular dimensions is about 30 Å.; furthermore, this is probably the shortest dimension of the molecule, since no smaller unit-cell dimensions occur. Physicochemical measurements are in agreement with the concept that the ribonuclease molecule can be approximated by a prolate spheroid with a ratio of about 1.2 between the major and minor axes. It is therefore reasonable to assume that the ribonuclease molecule is approximately a prolate spheroid with axial lengths about 30 Å. and 36 Å. The a and b axes of ribonuclease II are 30.28 Å. and 38.39 Å., respectively, which suggests that this crystal contains layers of molecules that

are centered at the lattice points of the lattice planes parallel to the *a* and *b* directions (the 011 planes), the short and long dimensions of the molecules above and below this plane, the shaded circles indicate molecules half way between the major and minor axes. It is reasonable, therefore, to assume that the ribonuclease molecule is approximately a prolate spheroid with axial lengths about 30 Å. and 36 Å. The *a* and *b* axes of ribonuclease II are 30.28 Å. and 38.39 Å., respectively, which suggests that this crystal contains layers of molecules that are centered at the lattice points of the lattice planes parallel to the *a* and *b* directions (the 001 planes), the long and short dimensions of the molecules being parallel to the *a* and *b* axes, respectively. The symmetry of this crystal requires that the unit cell contain two molecules related to one another by a twofold screw axis. Taking this into account, and placing the second molecule in the cell according to this requirement, one finds that all of the contact distances between the molecules agree with the assumption of a prolate spheroid of dimensions 30 Å. by 38 Å. A view down the *b* axis of this structure is shown in FIGURE 1. Each molecule appears circular in this projection; the open circles indicate molecules in the plane of the paper and multiples of 38.39 Å. above and below this plane, the shaded circles indicate molecules in planes half way between. The distances between shaded and unshaded neighbors are all equal to 34.1 Å. The unit cell and symmetry of ribonuclease II are thus completely compatible with the assumption of molecules shaped roughly like prolate spheroids with principal diameters of about 30 Å. and 38 Å.

It is easy to show that modifications I, V, and VII are also compatible with this same shape. FIGURES 2, 3, and 4 illustrate the probable packing of the ribonuclease molecules in each of these crystalline forms. The molecules are indicated by circles, but they should perhaps be shown as ellipses, since the spheroids may be tilted from the direction of viewing they probably are so tilted in types V and VII.

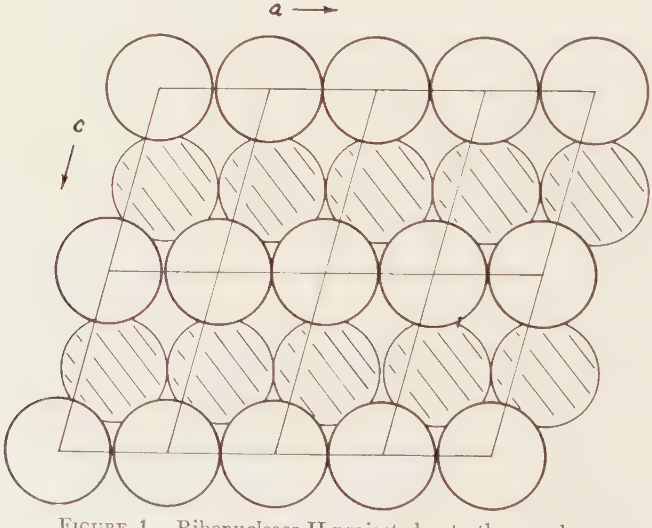

FIGURE 1. Ribonuclease II projected onto the *a*-*c* plane.

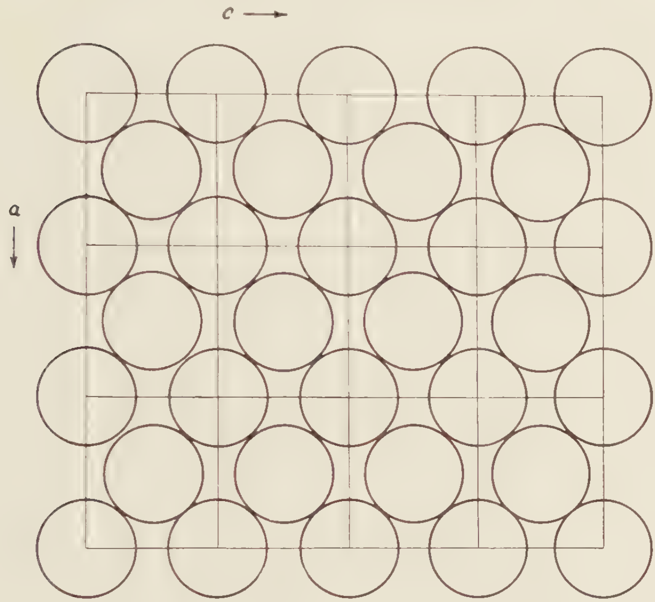

FIGURE 2. Ribonuclease I projected onto the *a-c* plane.

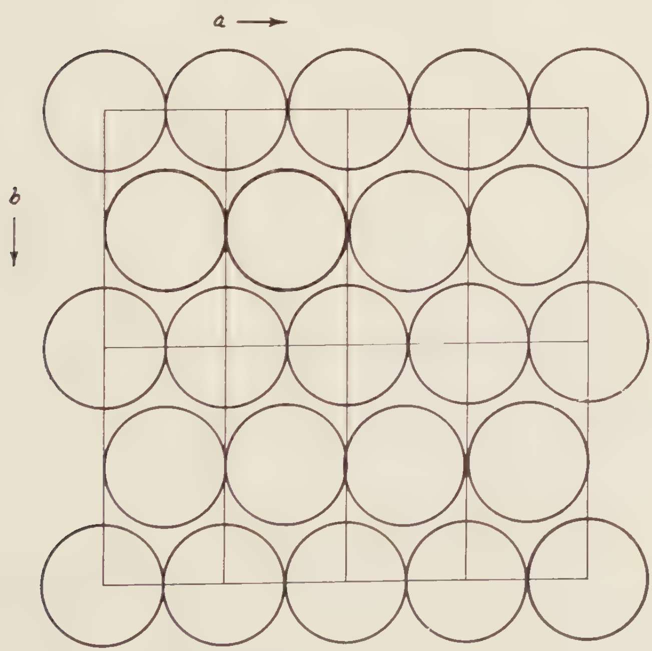

FIGURE 3. Ribonuclease V projected onto the *a-b* plane.

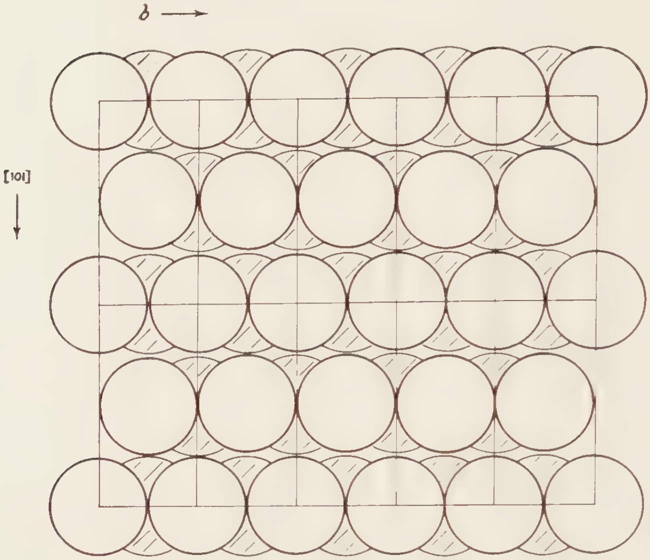

FIGURE 4. Ribonuclease VII projected onto the $10\bar{1}$ plane.

Further evidence in agreement with the concept that the ribonuclease molecule has this general shape is provided by measurements of the solvent contents of the various crystal modifications. In each case, the solvent associated with the protein is just about sufficient to fill the space in the crystal not occupied

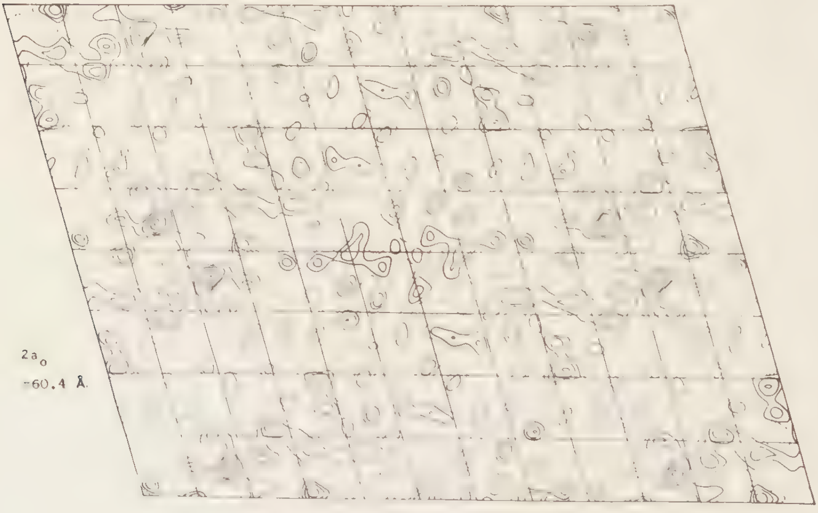

FIGURE 5. Ribonuclease II. Electron-density projection on the 010 plane. The axis b_0 = 38.4 Å. is perpendicular to the drawing. Resolution = 3Å.

by prolate spheroids 30 Å. × 38 Å. It is true that any other shape of molecule with the same volume will agree with this observation, but the fact is that this actually is the volume of the ellipsoid that corresponds to so many other data.

What information have we concerning the internal structure of the molecules of crystalline proteins? At the present time there is almost no genuine knowledge of the subject, but there has been a great deal of not unreasonable speculation based on somewhat arbitrary interpretations of the X-ray data. The reason for this indefinite situation is the fact that the X-ray diffraction data (the intensities of the thousands of rays diffracted by a protein crystal) cannot be used directly to compute the atomic arrangement in a crystal. From each experimentally measured intensity can be found directly a number $|F|^2$ which is related to two structurally significant numbers A and B by the equation $|F|^2 = A^2 + B^2$, and it is these last two numbers that must be known for each intensity in order to calculate the structure. It is possible to use the intensities diffracted from several isomorphous crystals in computing the A's and B's, provided that, in each crystal, the atoms of the protein occupy the same positions while the different crystals contain additional atoms of large scattering power arranged in different known ways. Work along these lines has been started in several laboratories, including our own, and is almost certain to succeed within the next few years, but at present, as mentioned above, the work is not sufficiently advanced to provide definite structural information.

FIGURE 5 shows the most definitive result so far obtained. It is a projection of the electron density in a crystal of ribonuclease II onto one of the faces of the unit cell. The resolution of the method was 3 Å., so that no details as small as atoms can appear in the figure. The unit cell in the direction of projection is 38 Å. thick (about 20 atomic diameters) and there is, consequently, a great deal of overlapping in the projection. All that can be said concerning the structure, on the basis of this figure, is that there is no evidence for parallel chains in the ribonuclease molecule such as have been postulated for other globular proteins. It seems more likely that the polypeptide chain in the molecule is folded into a complicated tangle. In order to discover the nature of the structure, it will be necessary to study the three-dimensional pattern of electron density—projections will not do. This three-dimensional study is now under way.

In conclusion, I should like to thank the various institutions that, by their continued support, are making possible the work of the Protein Structure Project. They are: The Dean Langmuir Foundation, The Rockefeller Foundation, The Damon Runyon Memorial Fund, Inc., The New York Foundation, Polytechnic Institute of Brooklyn, and International Business Machines Corporation, all in New York, N. Y.

SPONTANEOUS GENERATION OF ANABOLIC PATHWAYS, PROTEIN, AND NUCLEIC ACID*

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Modern ideas of spontaneous generation include a shift in emphasis from current organisms to the origin of the first organism. The historical attempts to duplicate a presumed natural spontaneous generation failed for at least one essential reason; namely that the experimenters were unaware of the long and intricate trail of Darwinian evolution. Studies of the production of maggots, mice, and microbes were stimulated by the sudden spectacular appearance of these organisms in the midst of decay, rather than by informed selection of the most meaningful organismic type. The modern concept is that life emanates from life, not from death, and that the first life arose from a sterile chemical world.

This proposition suggests at least two approaches to experiments that deal with spontaneous generation. One may experiment with hypotheses of the primordial event, or events, by looking forward from an inferred prebiological geochemistry. One may also look backward to that event by extrapolating in reverse our knowledge of biochemical evolution. It is, of course, true that a combination of these approaches to the primordial events offers the most hope. Although the problem of developing an adequate theory of spontaneous generation appears to be a very complex one, its solution should benefit from the fact that experimental results can be judged for both their prebiological and biological significance. The design and interpretation of experiments are thus simultaneously subject to the disciplines of chemical, biological, and geological points of view.

The initial study of this problem in our laboratory involved backward extrapolation of Darwinian evolution at the molecular level. This approach provided one explanation for microheterogeneity of protein and revealed that amino acids, proportions of amino acids, and sequential arrangements of amino-acid residues in the proteins of all organisms studied are much alike.[1] *N*-Terminal lysine was found to characterize the unfractionated proteins of each of eight plants tested.[1] The unexpectedly great similarity of protein molecules reflects an exceedingly slow molecular evolution. This interpretation encouraged a search for primordial protein and its production. The great diversity in organisms could be explained as due to an exponential number of macromolecular interactions[1] (TABLE 1).

Among many other consequences of these interpretations it was possible to predict that protein structure, when sufficiently understood, would present a picture of small essentially stepwise differences between molecules.[2, 3] This

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TABLE 1
THE EMERGENCE OF DIVERSITY

	Molecules*	Macromolecules	Organisms
	Matrix: 18 Amino acids; 6 Pyrimidines; 12 Vitamins; 6 Monosaccharides; 12 Mineral elements. Mainly composed of substances formed by loss of water	Proteins; Nucleic acids; Polysaccharides	Interactions of macromolecules; Nucleic acids and proteins, proteins and proteins, *etc.* Aqueous
Current degree of diversity	Limited diversity	Intermediate diversity	Extensive diversity

* Numbers of each type of molecule in matrix are minimal.

relationship has received support from the accumulated results of studies of oxytocin-vasopressin,[4] proteins of tobacco mosaic viruses (TMVs),[5] insulins of different species,[6] and melanophore-stimulating hormone (MSH)-adrenocorticotropic hormone (ACTH).[7]

When such studies led to experiments with origins of the biochemical world, several working premises were adopted. Two of these may be mentioned. One is that the origin of biochemical phenomena and that of life are not necessarily identical. It may well be that the chemical momentum of the correctly chosen processes has led, and perhaps can lead again, into the physiological and cytological aspects of life. On the other hand, it is also likely that we may be too ignorant to be aware of what is involved in the extrapolation, through natural processes, of the prebiological matrix to life itself. The other premise is that we must understand the generation of protein and metabolic pathways simultaneously. Biochemistry is characteristically taught and understood as a complex of enzymes (proteins) and metabolism. Quite apart from this relationship, these experiments began with the smaller, yet still considerable problem of the spontaneous generation of protein. It was and is believed that such a study might yield clues as to the origin, structure, and biosynthesis of these molecules.

For this study a temperature was chosen sufficiently high to overcome the thermodynamic infeasibility of forming the peptide bond, the reaction being driven by the removal of water.[8] The energy requirement for a dipeptide falls in the range of 3000 to 4000 cal. (TABLE 2). The qualitative fact that reason-

TABLE 2
FREE ENERGY REQUIRED TO FORM THE PEPTIDE BOND[9]

Reaction	ΔF°_{298}
Alanine + glycine = alanylglycine + H_2O	3730 cal.
Glycine + glycine = glycylglycine + H_2O	3230
Leucine + glycine = leucylglycine + H_2O	2960
Benzoic acid + glycine = hippuric acid + H_2O	2260

able terrestrial temperatures[10, 11] could form peptide bonds has been known for a long time (FIGURE 1). Such temperatures are found in volcanic regions even today.

The initial experiments designed to explain the origin of protein[13] consisted of pyropolymerizing one or two amino acids. These reaction products revealed many new ninhydrin-reactive components. Heating the ammonium salt of the Krebs Cycle acid, malic acid, yielded not only aspartic acid, but alanine. Aspartic acid yielded α-alanine and β-alanine. When urea was substituted for ammonium ion, the nucleic acid biointermediate, ureidosuccinic acid, was the unexpected result. For the most part the amino acids formed were not obtained as such, but only after hydrolytic treatment of the sort applied to proteins. Experimental evidence for many of the steps has been presented in the literature.[8] Preliminary evidence for other thermal products (FIGURE 2) has been obtained.

All of the results so far achieved can be summarized by three generalizations:

(1) Many staples of biochemistry can be formed by heating a few selected ones under chosen conditions. Nearly all of the products so far identified are recognizable as biochemical substances.

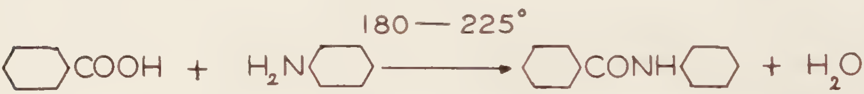

FIGURE 1. The formation of a "peptide bond" at anhydrizing temperature.

(2) The thermal sequences that produce these staples show an arresting degree of parallelism to anabolic pathways. Now that the evidence for this generalization has appeared, it can be asserted that Haeckel's Law ("ontogeny repeats phylogeny") should be obeyed at the biochemical level as well as at the biological level.

(3) The thermal pathways suggest simultaneous generation of metabolic sequences and of protein enzyme. This relationship is consistent with the biochemical emphasis on the interweaving of enzymes and metabolism. Furthermore, the simultaneous development of the material basis for inheritance of the reactions is suggested by the appearance of ureidosuccinic acid.

It should be noted that the thermal experiments have led to α-alanine in three ways and that Miller[14] and Abelson[15] each produced α-alanine in other ways, all of which also can be related to knowledge of primordial conditions. Amino acids are relatively stable organic compounds thermodynamically, due to their inner-salt (zwitterionic, dipolarionic) structure. They are energy-rich as compared to the elements or to the simple compound gases such as ammonia or carbon dioxide, but they are energy-poor as compared to many organic compounds. It should not be surprising, therefore, to find that any one of a number of sources of energy that act on a variety of gases, liquids, or solids that contain the necessary atoms should yield amino acids. Indeed, it would be surprising not to find them.

It is arresting, however, to observe the parallelism between the thermal path-

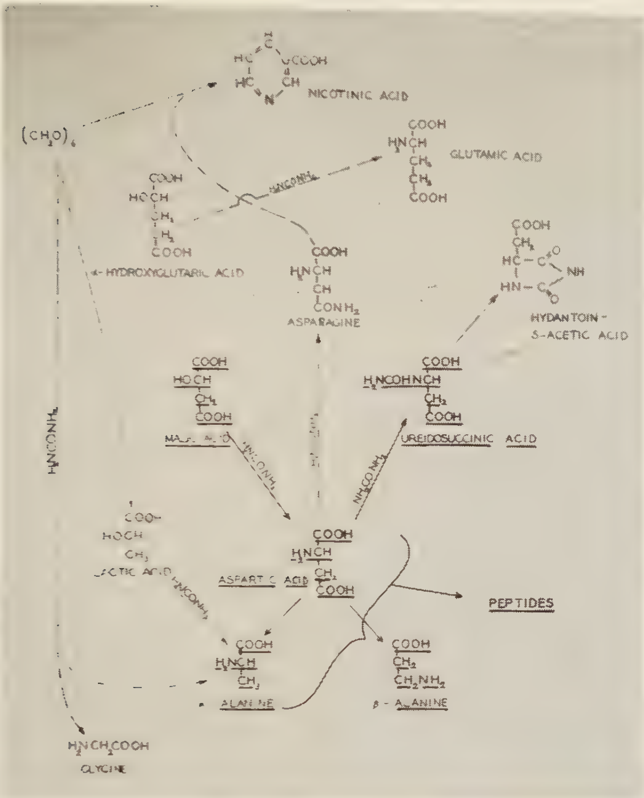

FIGURE 2. Thermal pathways with discernible relationship to biochemical pathways. Evidence for the reactions shown in underlined letters and unbroken arrows is presented in this paper or in an earlier one.[8] The reactions shown with broken arrows are from the literature or from experiments that have not yet been fully described.[12]

Asparagine is formed from aspartic acid and excess urea, and it is identified by paper chromatography. Nicotinic acid activity has been observed in repeated microbiological assays of glucose heated with asparagine. The conversion of glucose to lactic acid is described in the literature. Lactic acid is convered to α-alanine by heating with urea, and it is identified on paper chromatograms. The heating of glucose with urea yields spots for glycine and alanine in chromatograms that utilize three different solvent systems.

Urea is seen to participate in many of these reactions. It will be of interest to determine if urea can be substituted by the related carbamyl phosphate in some of the reactions.

ways and anabolic sequences. It is even more striking to find a large proportion of these reactions approximately described in the early literature of organic chemistry. The similarities may be merely the expression of an ability to find, in an abundant literature, evidence which conforms with a grand scheme. In the light of Haeckel's Law, however, the common nature of anabolic pathways and the studies of early organic chemists is due to a natural relationship. Spontaneous generation and simple organic experiments almost necessarily would begin with some of the same compounds, the range of which was limited by what was available terrestrially.

TABLE 3

BIURET TESTS OF POLYMERS FROM HEATING AMINO ACIDS AT 200°C. FOR 3 HOURS

Amino acid(s)	Biuret Test
Aspartic acid	+
Leucine	−
Phenylalanine	−
Valine	+
Phenylalanine-valine	+
Phenylalanine-aspartic acid	+
Valine-aspartic acid	+
Phenylalanine-leucine	−
Leucine-aspartic acid	+
Glycine[16, 17]	+

These data are from the work of Mavis Middlebrook, except as noted for polyglycine.[12]

The biuret tests were performed on the products after dialysis and washing with acetone. The test for polyvaline was not intense. It is of interest to note that the one negative test on a copolymer concerned amino acids which yield biuret-negative reaction products independently.

In an examination of the polymers formed thermally, initial results indicated positive biuret tests for most of the samples (TABLE 3).

Attempts to characterize the partially purified polymers in a quantitative manner encountered difficulty due to the fact that amino acids tend to form diketopiperazines (piperazinediones, cyclodipeptides) as well as linear peptides on heating to about 200° C. Recorded characterizations of amino acid polymers often have ignored the possibility that various analytic treatments might yield erroneous results through hydrolysis of the relatively labile piperazinedione structure (FIGURE 3). Recently, however, Meggy[16] has included analysis of such ring structures in his studies of polyglycine.

One line of evidence was obtained from glycine polymerized with urea and 85 per cent phosphoric acid for 60 hr. at 140–150° C. in an open tube. After dialysis followed by partial hydrolysis, chromatography revealed two spots with the R_fs of glycine-glycylglycine and of diglycylglycine. Glycine peptides are known to yield decreasingly intense ninhydrin reactions as the higher peptides are tested, until no visible reaction is observed above the tetraglycylglycine.[18]

In FIGURE 4 are presented the infrared tracings of pertinent materials. The pattern from one preparation of leucine-phenylalanine pyropolymer is almost identical with that of the diketopiperazine (DKP) from phenylalanine. This agrees with the negative biuret tests reported in TABLE 3. Other preparations of leucine-phenylalanine displayed what may be significant differences from that of this one preparation. The need for caution in interpretation of

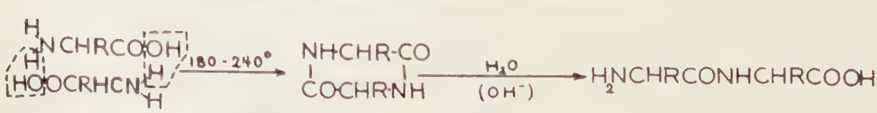

FIGURE 3. The thermal production of diketopiperazine and hydrolysis to peptide.

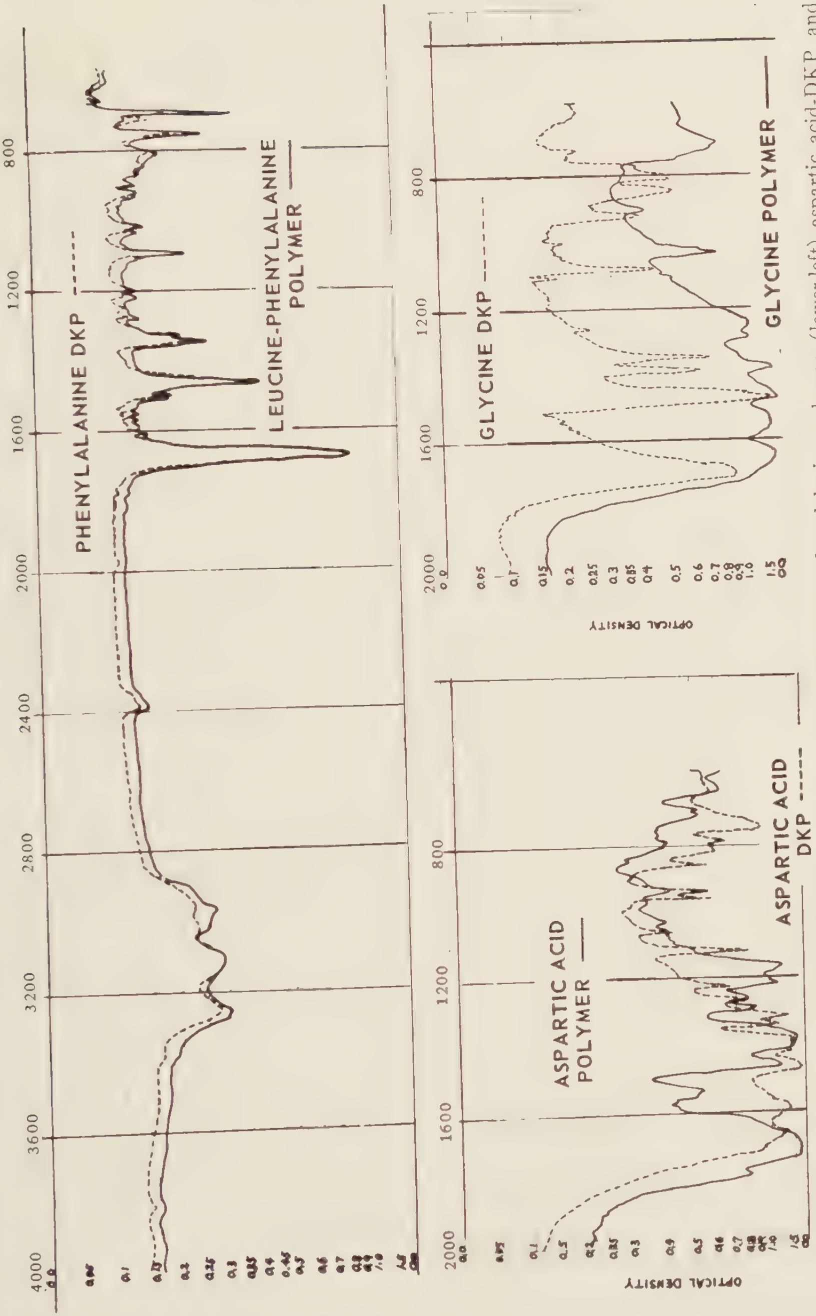

FIGURE 4. Infrared tracings of (top) phenylalanine-DKP and leucine-phenylalanine polymer, (lower left) aspartic acid-DKP and polyaspartic acid, and (lower right) glycine-DKP and polyglycine.

TABLE 4

RESULTS OF BIURET TESTS OF POLYASPARTIC ACID AND RELATED COMPOUNDS

Compound	Visual color	Copper complex spectral absorption maximum, mμ.
Aspartic acid	Faint blue	Diffuse
Glycylglycine	Blue	625 to 650
Glycine DKP	Blue	620 to 640
Aspartic acid DKP dimethyl ester	Blue, slowly	630
Aspartic acid DKP	Blue	630
3 Glycylglycine:1 polyaspartic acid	Blue-violet	570 to 590
Asparagine	Blue-violet	560 to 580
Valylglycylphenylalanine	Violet	555 to 565
1 glycylglycine:3 polyaspartic acid	Lilac	545 to 565
Polyaspartic acid	Lilac	540 to 550

The dimethyl ester evidently slowly hydrolyzes to the DKP in the alkaline biuret reagent. The results with the two mixtures of glycylglycine and polyaspartic acid indicate that the test may be adapted to semiquantitative analysis of mixtures of linear polypeptide with other compounds.

structure is emphasized, however, by the fact that one thermal polymer is essentially a DKP.

When the infrared patterns of glycine-DKP and of polyglycine (by heating glycine alone) are compared, it is clear that these are different substances. The same is true for aspartic acid-DKP and polyaspartic acid.[19]

The preceding information makes the significance of biuret tests clearer. Tests of key compounds, with the spectral absorption maxima of the copper complexes, are presented in TABLE 4.

It was particularly essential to prepare and test the aspartic acid-DKP, inasmuch as aspartic acid residues are known to shift absorption maxima of peptides toward the red,[20] as is true in the absorption spectrum of asparagine. Asparagine can be considered to be a dipeptide, but it yields a partly violet biuret test, even though a tripeptide is generally considered to represent the minimal structure for a biuret-positive substance. The failure of aspartic acid-DKP to yield a biuret-positive test, in contrast to polyaspartic acid, therefore is critical evidence that the polyaspartic acid is not merely the aspartic acid-DKP opening easily to behave like a linear peptide.

From these studies it is inferred that some amino acids such as leucine and phenylalanine do not homopolymerize to linear peptides under the thermal conditions that were employed. Others, such as glycine and aspartic acid, do lead to linear peptides when heated alone. Recent experiments indicate that the amino acids that do form linear peptides can modify the behavior of those that do not. One such experiment involves glutamic acid and glycine. It is well known that, when glutamic acid is heated alone, the inner lactam, α-pyrrolidonecarboxylic acid, results. When glycine is heated alone, a highly insoluble polyglycine results. When the two are heated together in equimolar amounts at 170° C. for one hr., there results a largely soluble product that gives an intense biuret test, in contrast to the α-pyrrolidonecarboxylic acid. Upon dialysis, hydrolysis, and chromatography, one finds intense spots for both

TABLE 5
SUGGESTED COMPOSITION OF PRIMITIVE ENVELOPE OF EARTH

Envelope	Author
CH_4, NH_3, H_2O, H_2	Oparin,[21] Urey[11]
Atmosphere CH_4, NH_3, H_2, H_2S Hydrosphere CO_2, NH_3, H_2S, H_2O	Bernal[22]
CO_2, NH_3, H_2S, H_2O	Rubey[23]
CO, CO_2, NH_3, H_2S, H_2O	Revelle[25]

glutamic acid and glycine. New possibilities in peptide synthesis now can be investigated. The provision of an answer to the problem posed by some amino acids accords more serious consideration to the thermal mode as a mechanism for the simultaneous emergence of anabolic pathways and protein.

The kind of chemistry that is revealed by the thermal studies may be compared with the kinds of atmospheric and hydrospheric envelopes inferred for the primitive earth (TABLE 5). Large gaps in our knowledge of the steps between primitive gases and the initial experimental thermal reactants remain to be filled. The formation of amino acids by electric or other means is a concept that is not incompatible with the thermal production of proteins. The emphasis on the carboxylic intermediates of the Krebs Cycle, however, seems to be most consistent with the carbon dioxide-rich systems of Bernal,[22] or Rubey,[24] and of Revelle.[25] By incorporating the reactive carbon monoxide of Revelle into Bernal's hydrospheric matrix, one can more easily visualize how the methylenic staples of organic and biological chemistry originated. This picture agrees with the emphasis of Darwin, Huxley, Bernal, and others upon the primitive ocean. A heated ocean, or tide pool, or hot spring represents a modification that comports better with the thermal evidence. A yet more consistent picture is the intrusion of a hot organic magma, formed under pressure, into marine waters, as through a volcanic fissure in the floor of the ocean.

Because of recent reports, at least one possible mode other than that presented here can meet the requirement of simultaneous spontaneous generation of protein and metabolic pathways. This is the Oparin-Urey-Miller scheme, as amplified by Otozai and his co-workers,[26] who found that "some sort of polymer of glycine" resulted from electrical discharge in glycine, although Miller did not report biuret-positive products in his first experiments.[11] As yet, however, a discernible parallelism between (1) the pathways of production of biochemical staples by nonthermal means and (2) biosynthesis is not evident.

Fundamentally, the need to solve the critical problem of the formation of protein stems from the need to understand the origin of enzyme. The problems thus brought to light, however, emphasize a more fundamental role for enzyme than that of a catalytic agent. As the thermal picture has developed it has become increasingly clear that a mechanism for trapping and repeating the component reactions was needed at the beginning for life to continue. The fulfillment of this need can be said to have marked the origin of the first life, or the emergence of life from its chemical matrix can be defined arbitrarily by this condition. With a possible thermal origin for protein and for nucleic acid,

one may visualize an answer with the aid of the Beadle concepts[27] of the relationships of reactions, enzymes, and genes. From this point of view, enzyme protein has functioned as an essential link in the material mechanism for trapping and remembering anabolic reactions. The role thus suggested is one to which the catalytic powers would be contributory, both originally and currently.

Enzymes often have been credited with speeding reactions without initiating them. When one recognizes that, without enzymes, many reactions proceed with immeasurable slowness, it can be seen that enzymes, *in effect*, initiate reactions. A combination of the Beadle concept and of the thermal theory then provides us with a material basis for trapping, storing, and somehow releasing the spontaneously generated chemical reactions. This concept emphasizes the significance of the discovery of ureidosuccinic acid, a biochemical intermediate for gene substance, as a product of thermal experiments in this framework. These interpretations also lead to the suggestion of a primitive mechanism for memory at the chemical level. The picture is one in which the thermal reactions occur in the presence of enzyme protein being formed simultaneously so that each reaction is impressed on the material of the enzyme. The enzyme thus formed is essentially trapped by nucleic acid until it is subsequently released by some trigger.

In this light, spontaneous generation would appear to have occurred by a transition from chemical simplicity through complexity of a type that led to its own essential repetition. Once launched, life could undergo Darwinian evolution at least to the point at which the product might understand the entire process that stems from the spontaneous generation of a chemical memory.

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THE ROLE OF THE GENE IN EVOLUTION*

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Precellular Life

Oparin[1] conceived of life as arising from a sea (or in tide pools) rich in organic material. He proposed that molecules aggregated in coacervates became associated into organizations that tended to increase their peculiar types of aggregation in volume and in quantity by the consumption of simpler molecules and by means of the energy supplied by the energy-rich milieu. Although he published the theory in 1936, Oparin did not mention genes, and one gains the impression that he conceived of the independent origin of "nucleus, chondriosomes, protoplasts and other organoids"; he conceived of the origin, multiplication, and struggle for survival between all imaginable types of bionts. Allen[2] has proposed that there probably were "self-duplicating, mutable systems of simpler chemical constitution than nucleoproteins" in the precellular stages of evolution.

Oparin recognized the importance of enzymes in conferring competitive advantage in natural selection, but he did not consider them "primary" requisites. In his opinion, life originated by coacervation of materials that facilitated their own synthesis by their peculiar structural coordinations under conditions in which the chemical reactions involved occurred at unaccelerated rates. Oparin suggested further that devices that controlled the synthesis of enzymes were incorporated subsequently. Yeast cells possess specific genes that control the synthesis of enzymes for the splitting of specific saccharides, and yeasts that possess the ability to produce these enzymes thereby have specific advantages over other yeasts and other microorganisms that lack them. However, if time were unimportant and if competition were negligible, the saccharides eventually would break down and become available spontaneously; in a noncompetitive situation the cell would do as well without such genes as with them. Thus, the advantages conferred by genes that control the synthesis of the enzymes are apparently secondary, depending upon competitive conditions.

The Primacy of the Gene

Muller[3] is the chief proponent of the primacy of the gene. In discussing the origin of life, Muller quotes Oparin without pointing out that their views are fundamentally different. According to Muller, "In the origination of life the gene arose first, and . . . protoplasm came into existence later, very gradually, in the form of a series of products of the chemical action of aggregates of genes that had mutated in such ways as to be able to give rise to these products. Protoplasm would thus consist of substances accessory to and produced by the genes. Its existence would be due to the fact that those mutant genes had been naturally selected whose products happened to afford chemical tools, such

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as enzymes, that are useful for the survival and multiplication of these genes themselves."

Muller makes it quite clear that he dates the origin of life very precisely. He states, "And if, among the myriad types of molecules thereby produced, genes were included (*only one successful gene being required!*),* then the component parts also would already have been formed, out of which these genes could manufacture duplicates of themselves. Moreover, there would also be numerous other ready-made constituents present, which were capable of being utilized as accessory substances after mutations implementing such utilization had occurred in the descendant genes." In the opinion of many geneticists who agree with Muller, the gene is the unique "living" particle that is characterized by (1) the ability to reproduce itself, (2) the ability to undergo variation or mutation, (3) the ability to reproduce the variation as faithfully as it produced the original form, and (4) the ability of both the original form and the variant to produce specific substances that control cellular metabolism. The most recent distillation of biological thought on this subject is summarized by the slogan "one gene—one enzyme." An historical review may help to understand the development of this opinion.

The theory of evolution was developed before gene-controlled heredity was demonstrated. Genetics began to advance with startling rapidity after the rediscovery of Mendelism, and many enthusiasts were convinced that the gene was specifically different from any other cellular component or organelle. This view was fostered by the evidence that evolution seemed to depend largely on the mutation and the redistribution of genes. Many embryologists held, however, that the gene was a relatively trivial part of the hereditary apparatus that accounts for differences between the races of a species or the species of a genus. They proposed that the fundamental characters (such as those distinguishing classes or phyla) were controlled by the "cytoplasm." This attitude led geneticists to overemphasize the importance of the gene, and extremists contended that the gene was the "fundamental living particle." Whether life originated by the origin of a single gene (as Muller suggests) or by the parallel origin of nuclei, cytoplasm, and other organelles (as Oparin proposes), it is abundantly clear that nucleus and cytoplasm are hopelessly interdependent at present.

Definition of the Gene

Before considering the nature of the gene it is necessary to define it. It has always appeared to me that the most precise, succinct, and complete account of modern genetics is Sturtevant and Beadle's *An Introduction to Genetics.*[4] It is of interest to know that the gene is not defined in this remarkable volume. It is even more informative to know that, after the publication of this work, its authors found that each of them thought of the gene in a different way. One of them considered it primarily as a location, and the other as a functional unit. Consequently, some temerity is required to attempt the following definition:

The normal gene is inferred to be a particle (part of a continuum) located on

* My italics. C. C. L.

a chromosome and is identifiable only if at least one defective allele of it exists; that is, genes are detectable only in pairs. The heterozygote (normal/defective) produces sex cells that are distinguishable (again by inference) through the manner in which normal and defective offspring are distributed among the offspring of the hybrid, on the assumption that a normal gene produces a normal offspring. The location of the gene pair is determined by the frequency with which members of a given pair recombine with members of other gene pairs. The definition of any gene is always tentative, since the gene is a unit that is defined by recombinations that are inferred to result from crossing over. The gene is defined in terms of recombination *due to crossing over* because recombination may result from mechanisms other than this, and crossing over must be inferred from the observation of recombination (failures to distinguish between recombination and crossing over are found in nearly all texts on modern genetics). All of this leaves a degree of ambiguity in the definition of any gene, since recombination due to crossing over cannot be established positively except by tetrad analysis. This ambiguity generally is ignored in bacterial and viral genetics. When a supposed gene is found to be complex (that is, capable of being broken up into smaller units by recombination due to crossing over) it must be redefined.

Demerec[5] has analyzed the problem of the gene (using *Salmonella*) in a study in which the ambiguities referred to above play an important role. If one accepts all of his assumptions it may be inferred that he is working with genes, but on the above definition Demerec has designated as a "*site*" what I would call a "*gene*". What he calls a "single gene" is actually a "*cluster*" of genes. Demerec's analysis only postpones consideration of the problem, since the fundamental problem dealing with the mechanism of gene action is not different from that connected with the mechanism of site action.

The Apparent Autonomy of the Mutant Gene

The normal gene does not and cannot reproduce itself; the gene is reduplicated by a system (the cell) of which the gene is only a part. No chemical possesses the capacity for self-reproduction; it must depend upon the environment for its components and, if the synthesis is to be rapid it must be mediated by an enzyme. Self-duplication is reducible to a mechanism whereby the enzymes that control the synthesis and the structure that is synthesized are associated intimately. The defective nature of a cell that carries a mutant gene is caused by the absence, diminution, or deviation of the contribution ordinarily made by the normal gene. The defect becomes apparent because the mutant cell produces sufficient material to enable it to reproduce without the contribution previously made by the normal gene, but in a characteristically different, possibly defective, manner. The altered condition of the defective mutant is due to the defect in the chromosome at the location technically known as the "mutant" gene. This phenomenon has led to the view that genes are structures that differ from other cellular components by their specific ability to *reproduce variations of themselves* as if the mutant gene were a "new" gene that produced something new and different. This is fundamentally incorrect. It is correct, however, to say, that, when a defect or deletion occurs

in a small segment of a chromosome, the rest of the organism can continue, albeit in a changed condition.

During evolution changes may occur more frequently in the chromosomes than in other organelles, simply because chromosomes can be altered with fewer ill effects than when any other permanent structure is modified. The chromosomes differ from the other permanent organelles in their high degree of linear heterogeneity. Mutations are relatively minute defects or deletions in the extraordinarily heterogeneous chromosomes, each of which comprises only a tiny fraction of the genome. Normal growth cannot be achieved unless the chromosomes, with their highly heterogeneous linear structure, are transmitted from cell to cell in a regular manner, since normal growth requires a complete set of genes.

The Possible Autonomy of Cellular Organelles

Direct examination of cells suggests that many organelles other than the chromosomes are as autonomous as are chromosomes. The capacity of the yeast cell for continuous growth may be interpreted as the result of a specific structural association of the different orgnaelles that make the whole cell capable of producing all of the components necessary for the synthesis of all the cellular organelles when placed in an adequate milieu. Many of the organelles of the yeast cell appear to have the same integrity and continuity in time that characterizes chromosomes; they do not appear to arise *de novo*. Most of these cellular components divide in a manner that does not provide for precise transmission of specific portions to each daughter cell. Their failure to divide into precisely equivalent fractions suggests that they may be relatively homogeneous or, if heterogeneous, that their heterogeneity may be that of a simple coacervate. The cell can function only if all of its component parts are present in proper structural correlation and in adequate amounts. It maybe that none of the components (including the gene) is unique in the manner in which it reproduces itself; they all may reproduce by a similar type of accretion of molecules like those that they contain, and their association with each other in an adequate milieu provides the molecules necessary for their increase in size. If each of the permanent organelles is rate-limiting, when any one is present in less than the minimal amount the other organelles cannot obtain the supply of molecules necessary for maintenance and increase until the deficient organelle has been built up to the point where it can make an adequate contribution.

Driesch[6] argued that no mechanist could conceive of a mechanism (such as a watch) that could duplicate itself. His argument has weight when one considers mechanisms such as watches in which mechanical cogs mesh together by physical contact. If, however, the cogs are protoplasmic organelles that mesh together by chemical reactions, the problem assumes a different aspect.

The Possible Functions of the Nucleic Acids

The almost incredible advances in biochemistry in the past few decades have led to the optimistic view that genes are composed exclusively of deoxyribo-

nucleic acid (DNA), and that the secret of life lies in the structure thereof, however, since the time of the philosopher's stone, chemists have always expected more of chemical specificity than it possibly could deliver. A biological building block must be both sticky and rigid, and the importance of the nucleic acids may depend on these characteristics rather than on DNA-determined genic specificity. The critical evidence for the genic specificity of DNA is that DNAase destroys the transforming principle that is capable of inducing transformation in bacteria. This has been inferred to mean that the gene is composed exclusively of DNA. It is possible, however, that DNA is merely a structural element that holds together a coacervate that comprises the gene, and that DNAase produces its effect by the destruction of the DNA skeleton, and in this manner disassembles the coacervate. The argument that the action of the enzyme proves that DNA is the exclusive component of the gene is equivalent to the contention that, if removal of the skeleton will destroy an animal, then the skeleton is the animal. The primary plasmic masses of Oparin[1] might have been more effective in competition if provided with skeletons on which the chemical reactions could be organized on an assembly-line basis. This would permit much more efficient growth than the random chemical activity that is presumed to be characteristic of the primal plasmas.

The Origin of the Integrated Cell

Two principal kinds of bionts that depend on nucleic acids (one using ribonucleic acid (RNA), and the other, DNA) may have competed in the primal seas for survival with other kinds of coacervates that lack nucleic acids. The "cytoplasmic" bionts synthesized RNA and throve when uracil and ribose were in excess; the "nuclear" bionts synthesized DNA and throve when thymine and deoxyribose were abundant (FIGURE 1). If both depended principally upon external sources of these nutrients, an association between them, probably as loose as that characteristic of the components of lichens, would have survival value, although at times an excessive growth of one or the other phase might occur. As external sources become scarce, a mechanism for balancing the syn-

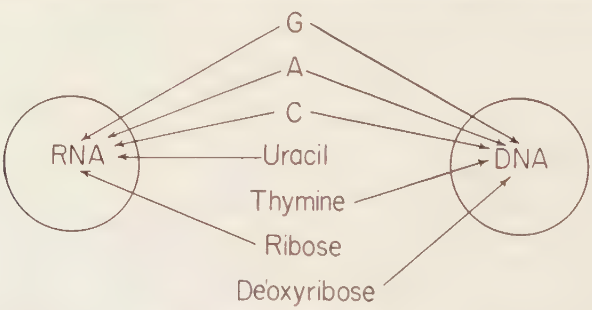

FIGURE 1. At least two kinds of bionts, distinguished by the DNA and RNA, which composed their respective "backbones," may have competed with each other in the primal tide pools. Both of them drew upon a common supply of guanine (G), adenine (A), and cytosine (C). The RNA bionts required uracil and ribose, while the DNA bionts required thymine and deoxyribose.

thesis of these components from simpler materials would confer an enormous advantage, and it may be this device that controls the karyoplasmic ratio in contemporary bionts.

It would be difficult to conceive of the original plasmic masses achieving the differentiation characteristic of the modern metazoan. This may have been achieved by the reduction of the size of the mass to microscopic building blocks of standard size, characterized by diphasic (nuclear *versus* cytoplasmic) growth. It has been known since Boveri[7] that the karyoplasmatic ratio is a constant. Recent experiments with yeast indicate that nuclear and cytoplasmic syntheses are competitive and mutually exclusive processes. Symbiosis between the DNA and RNA primal plasmas could have been made effective by the alternative and interdependent synthesis of DNA and RNA. The diphasic nature of growth could be the result of a mechanism that halts the synthesis of uracil and ribose and initiates the synthesis of thymine and deoxyribose and vice versa, thus making the synthesis of the respective "backbones" of the nucleus and of the cytoplasm interdependent and alternative (FIGURE 2). (Seymour Cohen[8] has provided a molecular model for one of these events.) Other kinds of plasmas could have been integrated into this effective cell as partial parasites or symbionts to produce a primitive cell from which the apparently monophyletic living organisms with which we are familiar originated.

Mutability and Loss of Extrachromosomal Organelles

If the different organelles are all equally autonomous it is possible to suppose that advances in evolution can occur by changes in the composition of any one of them. The condition for the perpetuation of any change would be that the variant organelle could be provided with the materials necessary for its continuance by the cell as a whole in its surrounding environment *at the time of the*

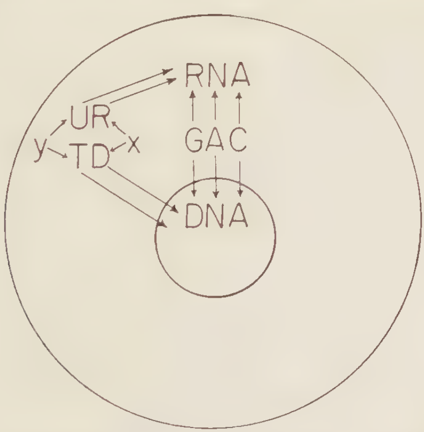

FIGURE 2. The karyoplasmic ratio. In the integrated organism, guanine (G) adenine (A), and cytosine (C) are supplied for either RNA or DNA synthesis. The diphasic control of growth and, consequently, of the cell, may be controlled by some mechanism that shunts the synthesis of a precursor *x* from ribose to deoxyribose, and that of a precursor *y* from uracil to thymine, and vice versa.

occurrence of the change. On this hypothesis each autonomous component of the cell has a potential for initiating changes in evolution. More changes may be observed in the chromosome than in the extrachromosomal organelles because of the large effect produced by a small amount of material in the heterogeneous chromosomes. The bulk of material in the other organelles would tend to overwhelm a single variant molecule that occurred in a coacervate even if it were advantageous and could be propagated. On this hypothesis the plasmone is more resistant to variation than the genome. The mitochondria appear to be somewhat heterogeneous, but only rarely are specific devices available to ensure their precise transmission. The cytoplasm is certainly heterogeneous, but it appears to be made up of small particles the transmission of which is insured by their large numbers. In the germ line, in addition to the chromosomal apparatus, the entire extrachromosomal potential must be maintained. The prime function of the germ line, on this hypothesis might be to maintain intact the *extrachromosomal* (rather than the chromosomal) apparatus. Genetic analysis of yeasts reveals numerous instances in which defects due to losses of autonomous extrachromosomal organelles are replaced by outcrossing.

Plasticity of the Gene

The concept of the stability of the gene is one of the fundamental principles that underlie the theory of the gene. According to Morgan,[9] "Mendel's theory of heredity postulates that the gene is stable. It assumes that the gene that each parent contributes to the hybrid remains intact in its new environment in the hybrid. . . . If a black guinea pig is bred to a white one, the offspring are black. If these are inbred, the offspring are three blacks to one white. The extracted whites breed as true as the original race of whites. The white gene has not been contaminated by the black gene in their sojourn together in the hybrid." The conclusion "that the white gene has not been contaminated" is an inference based on the assumption of a gametic ratio of 2:2 (AAaa from an A/a hybrid) at every meiosis. Direct evidence from tetrad analysis of heterozygous *Saccharomyces* hybrids (A/a) contradicts this inference. The direct evidence revealed four unexpected kinds of tetrads: AAAA, AAAa, aaaA, and aaaa. The irregular tetrads are of two types: those that contain an excess of normal (dominant) genes over the expected 2:2 and those that contain an excess of defective (recessive) genes over the expected 2:2. The phenomenon by which these exceptions are produced is called gene conversion.

Gene conversion comprises the interaction between alleles (A and a) in a heterozygote (Aa) that results in either the loss of capacity of a normal allele (A → a) or the restitution of capacity in a defective allele (a → A). This phenomenon is detectable only by tetrad analyses. Most of the data obtained from tetrad analysis have been consistent with the Mendelian concept, but many of the exceptions that have been encountered cannot be explained on any conventional Mendelian theory.

The MZ Gene in Saccharomyces

The most interesting gene thus far encountered is one that controls the fermentation of melezitose in *Saccharomyces*.[10, 11, 12] The formation of the *adap-*

tive enzyme, melezitase, occurs in this yeast when cells that carry the totipotent gene at the MZ locus are exposed to any one of five substrates. The five substrates are turanose (T), maltose (M), sucrose (S), methyl-α-D-glucopyranoside (G), and melezitose (Z). A convenient designation for this genotype is MZ^{TMSGZ}. The phenotypes can be designated by plus and minus signs as follows: T+/T−, M+/M−, S+/S−, G+/G−, and Z+/Z−. The multiple alleles of MZ can be designated by the superscripts alone; thus, the totipotent allele would be TMSGZ. The multiple alleles of this locus thus far observed are limited to the following: TMSGZ, TMSGz, TMSgZ, TMSgz, TMsgz, Tmsgz, and the recessive tmsgz. The TMSGz, TMSgZ, TMSgz, TMsgz, and Tmsgz strains ferment all five substrates after adaptation to an appropriate inducer. For example, TMSgz cells cannot use either methyl α-D-glucopyranoside or melezitose, but they do ferment these substrates after adaptation to turanose, maltose, or sucrose (FIGURE 3). Fermentation occurs in the absence of extensive growth, which excludes population shifts as an explanation of the phenomenon.

Competitive Inhibition by Inducers of Adaptive Enzyme Synthesis

Induction of melezitase by maltose in the TMsgz phenotype is competitively inhibited by sucrose (FIGURE 4). When TMsgz is exposed to maltose, adaptative fermentation occurs within 2 hours, and the fermentative activity that is induced by exposure to the substrate is maximal. Exposure of this culture to sucrose does not result in the fermentation of sucrose over the ten-hour period during which testing in the Warburg apparatus is feasible. When sucrose and maltose are mixed in the Warburg apparatus the fermentative ability achieved

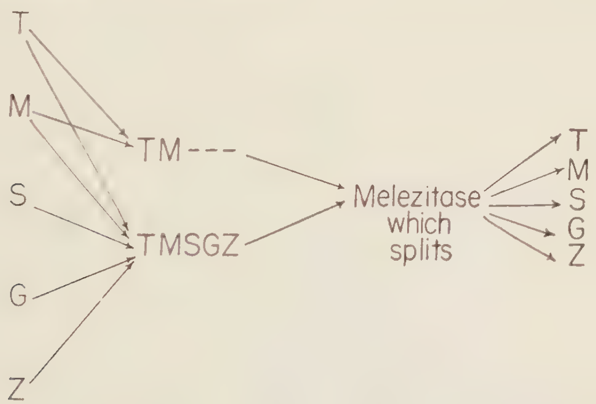

FIGURE 3. A diagram to distinguish the alleles TMSGZ and TM---.

The gene TMsgz is adaptively stimulated by............	either maltose or turanose (but not by sucrose or methyl-α-D-glucopyranoside or melezitose)	to produce the enzyme melezitase which acts on maltose, turanose, sucrose, methyl-α-D-glucopyranoside, and melezitose.
The gene TMSGZ is adaptively stimulated by............	either maltose or turanose or sucrose or methyl-α-D-glucopyranoside or melezitose	

by the TMsgz cell is much less than that invoked by maltose alone. This suggests that sucrose affixes to the surface of the gene and prevents contact with the surface by the maltose. It is clear that sucrose can enter the cell, since TMsgz that has been adapted to maltose ferments sucrose immediately, and thus excludes the possibility that permeability may be involved in the failure of TMsgz to adapt to sucrose. The fact that TMsgz is incapable of developing maximal capacity to ferment maltose in the presence of sucrose also suggests that they both enter the cell to compete for a surface. It is inferred that the deformation that changed TMSGZ to TMsgz did not change the surface sufficiently to prevent sucrose from attaching to it, but that the fit was not sufficiently precise to permit sucrose to function as an inducer.

Gene Conversion at the MZ Locus

When TMSGZ, TMSGz, TMSgZ, or TMSgz are mated to each other or to tmsgz, regular segregation of T+ M+ T− M− phenotypes occurs and, if only these two criteria were available, the gene would be considered as practically 100 per cent stable. However, multifarious variations in regard tc S+/S−, G+/G− and Z+/Z− are found; these reflect the effects of conversion (assumed to occur at meiosis) that result in the production of the following types: TMSGZ, TMSGz, TMSgZ, TMSgz, and TMsgz (FIGURE 5). It is important to point out that the occurrence of four negatives (that is, a TMSGZ × tmsgz hybrid produces a Z− Z− Z− Z− ascus, TMSGz TMSGz tmsgz tmsgz) means that the original dominant gene TMSGZ was modified in the

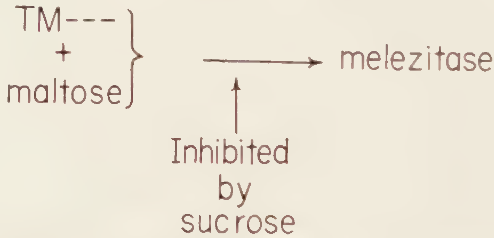

FIGURE 4. The adaptive production of melezitase induced by maltose acting on TMsgz is inhibited by sucrose. This indicates that the TMsgz locus can hold sucrose to its surface, although this locus does not respond to sucrose as an inducer.

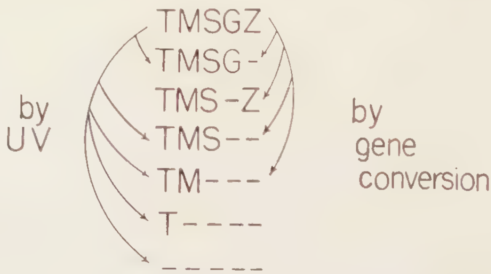

FIGURE 5. Types of alleles produced by gene conversion and by ultraviolet radiation. All of these alleles are stable in mitosis and generally are stable in meiosis.

zygote and that, in addition to changing the "new" genes, conversion altered one of the "old" genes in a parental chromatid from TMSGZ to TMSGz.

Pseudoalleles comprise closely linked genes that are separable by recombination due to crossing over. They are phenotypically similar, but two or more genes are required to produce the normal types. It was originally inferred that the TMSGZ complex comprised a single gene in which crossing over did not occur, since T+ M+ T− M− always segregated regularly in hybrids, while S+ S−, G+, G−, and Z+ Z− were distributed irregularly, but the T− M− individuals are always S− G− Z−, while the T+ M+ individuals were either S+ S−, G+/G−, or Z+/Z−. Members of individual tetrads would be:

T+ M+ S+ G+ Z+, T+ M+ S+ G+ Z−,

T− M− S− G− Z−, T− M− S− G− Z−, or

T+ M+ S+ G+ Z+, T+ M+ S+ G− Z−,

T− M− S− G− Z−, T− M− S− G− Z−, etc.

This indicated that the recombinations of S+/S−, G+/G−, and Z+/Z− were not due to crossing over. We considered the possibility that pseudoallelism might explain the phenomenon. The demonstration that no combination of nonfermenters could restore fermentative ability to the hybrids or to their progeny excluded pseudoallelism.

Orderly Degradation of MZ by Ultraviolet Irradiation

Irradiation causes losses of abilities to respond to the different inducers (FIGURE 5). Only the following 5 types have been obtained by ultraviolet irradiation of TMSGZ: TMSGz, TMSgz, TMsgz, Tmsgz, and tmsgz. The types most frequently induced are those with fewest losses. The abilities are lost in an orderly, regressive sequence.

Since only 5 phenotypes were induced by ultraviolet radiation of the totipotent phenotype, when 31 might have been expected if each character were controlled by an independent gene, the possibility that ultraviolet radiation induced recessive mutation of 5 independent genes is excluded. The alternative presumes that fermentation of turanose is controlled by one gene (A), maltose by 2 (AB), sucrose by 3 (ABC), methyl-α-D-glucopyranoside by 4 (ABCD), and melezitose by 5 (ABCDE), according to TABLE 1. Inactivation of the individual genes could lead to the observed array of phenotypes, but the

TABLE 1

Phenotypes	Hypothetical genotypes	Numbers
T+ M+ S+ G+ Z+	ABCDE	1
T+ M+ S+ G+ Z−	ABCDe	1
T+ M+ S+ G− Z−	ABCde and ABCdE	2
T+ M+ S− G− Z−	ABcde . . . ABcDE	4
T+ M− S− G− Z−	Abcde . . . AbCDE	8
T− M− S− G− Z−	abcde . . . aBCDE	16

expected *relative* frequencies of the different phenotypes on this hypothesis should occur in the reverse order to that which was observed experimentally (tmsgz > Tmsgz > TMSgz > TMSGz).

Damage by ultraviolet rays to the MZ gene is an intragenic phenomenon. The totipotent allele is inferred to comprise a "mirror-image" capable of conforming to the surfaces of at least 5 different substrates. The minimal radiation damage to the totipotent allele involves a change in configuration that preserves the capacity of the locus to "fit" 4 of the substrates (turanose, maltose, sucrose, and methyl-α-D-glucopyranoside), but renders it incapable of fitting melezitose with sufficient precision to permit the latter to function as an inducer of enzyme. The different deformations must correspond to the spatial relations that differentiate the structures of the different inducers.

Controlled Regeneration of the MZ Gene by Exposure to Substrate

Exposure to the inducers sucrose, methyl-α-D-glucopyranoside, and melezitose acts mutagenically to restore all the capacities of any deficient member of the series in a single step except tmsgz, while maltose effects only partial restoration (FIGURE 6). For example, the exposure of Tmsgz to melezitose regenerates the gene to the TMSGZ condition, while exposure to maltose only restores it to the TMSGz condition. This is the most convincing evidence yet available indicating that the gene is a plastic structure that can be remodeled by substrate and specifically remodeled by a specific substrate. If one supposes that the gene is a mirror-image of the substrate, then mutation from defective to normal induced by substrate might be due to remodeling of the surface of the gene by the surface of the substrate. The altered configurations of the MZ gene generally are stable during meiosis except for gene conversion. Gene conversion at meiosis from defective to normal might be the result of the transfer of gene material without crossing over from one locus to another. Gene conversion at meiosis from normal to defective could be the result of distortion of a normal gene surface by contact with the defective gene surface. The genotypes are stable during vegetative proliferation, except as pointed out above, when exposed to certain specific substrates.

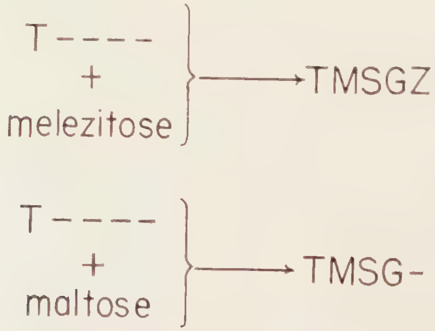

FIGURE 6. The regeneration of the Tmsgz allele by contact with substrate. Melezitose reconstitutes the allele to TMSGZ, while maltose induces TMSGz mutants only.

Regeneration of the Gene Mediated by X Rays

The tmsgz recessive allele was refractory to regeneration by any of the five substrates, and it failed to produce offspring of the fermenter type on any medium that contained these substrates. D. Pittman, in our laboratory,[13] recently has shown that if cells that bear the recessive gene tmsgz are treated with X rays and subsequently are exposed to melezitose, the completely regenerated gene (TMSGZ) is produced after exposure to melezitose (FIGURE 7). This important discovery reveals the fact that X rays have the interesting capacity to increase the plasticity of the gene and to facilitate and reinforce the mutagenic action of substrate. The significance of this phenomenon is considerable, since it suggests that genic material highly refractory to remodeling can be effectively altered if X-rays and substrate act on it jointly. This information may alter our views on the possible rapidity of progressive evolution.

Characteristics of the MZ Gene

The size of the exposed surface of the MZ gene on which the inducer rests cannot be much larger than a trisaccharide, although it may be replicated many times over the surface of the chromosome so that the splitting of the chromosome does not fail to transfer the pattern to both daughter strands. Many replications will leave the surface much smaller than are indicated by the largest present estimates of gene size. There is little reason to suppose that a DNA surface would be plastic, but rather that surfaces like those discussed by Pauling[14] in antibody synthesis may be involved. The multiple alleles and the enzyme controlled by them are not identical, since different alleles produce the same enzyme. The general structure of the cell and the relation of the of the nucleus to the cytoplasm strongly suggests that enzymes the production of which is initiated on the chromosome actually are constructed in the cytoplasm (since the nuclear membrane forms a barrier between the nucleus and the cytoplasm, in which most metabolic activity appears to occur). The

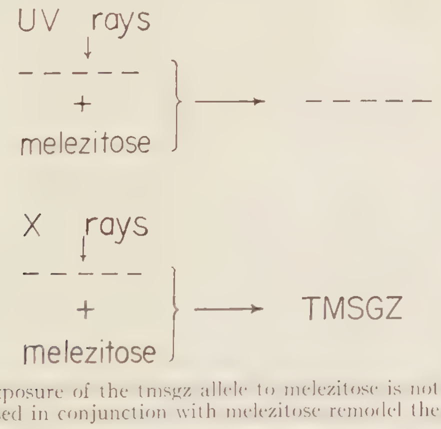

FIGURE 7. The exposure of the tmsgz allele to melezitose is not sufficient to regenerate the surface; X rays used in conjunction with melezitose remodel the surface to form the totipotent allele.

synthesis of the enzyme that is initiated by contact with the inducer is effected in a nonspecific manner; the same enzyme is produced by each of the five inducers. The surface configuration is a trigger that may be actuated by any one of five inducers to produce a stimulus that results in the production of melezitase. The synthesis of enzyme in the cytoplasm may be initiated by a specificity-conferring catalyst (one that contains RNA) which passes from the nucleus (presumably from the nucleolus) into the cytoplasm. The specificity-conferring catalyst may be synthesized at some distance from the surface on which the inducer impinges. The total mechanism may involve at least four components: (1) the trigger, (2) the path along which the stimulus travels, (3) a receptor which responds to the stimulus to produce the specificity-conferring catalyst, and (4) a mechanism in the cytoplasm which elaborates the completed enzyme.

The Appropriation of "Cytoplasmic" Activities by Genes

It is clear that the genes that control the splitting of carbohydrates in *Saccharomyces* confer advantages that depend upon competitive conditions, and that the cell could do as well without them if competition were negligible. These capacities have been localized and sequestered in the nucleus where they lie in wait for the specific conditions in which they will be useful. They are maintained in a prepared condition over countless generations. They constitute an insurance of success in conditions of competition, but they are not directly involved in the primary metabolism of living activity. Not all such capacities have been isolated in the nucleus. A good example is the ability of the yeast cell to respire oxidatively. This capacity resides principally in extrachromosomal structures that are readily lost by misdivision or inaction. There may be good reason why it would not be profitable, from the standpoint of survival or differentiation, to have control of this capacity built into the chromosomes; it may be that the conditions for anaerobiosis are so considerable among yeasts that the survival value of making it a permanent part of the genome is negative. Be that as it may, it is a capacity easily lost on misdivision, but one that can be restored in an outcross. It provides an excellent example of a capacity that *could* be transferred to a gene. The first step might be unequal crossing over that produces an extra length of chromatin with a duplicate (and therefore useless) function. This duplicated plastic material would next receive impresssions that lead to the initiation of a structure that controls the synthesis of the oxidative enzymes, and thus transfer the capacity from the cytoplasm to the nucleus. The nucleus probably has aggrandized its power over the rest of the cell by such a stepwise process, and in this manner it has taken over control of one vital activity after another. It is easy to understand how this transfer of control has led to greater efficiency, since unnecessary bulk is reduced and an enormous variety of capacities can be confined to a small space permanently available at any time the conditions may arise. On this basis, the gene is a secondary rather than a primary item in evolution and, although this organelle now appears to control a wide range of cellular activity, it seems clear that it has achieved this position by a creeping

bit-by-bit onslaught until the cell, which was originally independent of genes, has now become hopelessly dependent upon them.

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THE ORIGIN OF OPTICAL ACTIVITY*

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No other chemical characteristic is as distinctive of living organisms as is optical activity. Outside of organisms, all syntheses of disymmetric molecules produce equal numbers of optical antipodes (racemic mixtures) unless deliberate means are employed to bias the result by the use of asymmetric reagents or forces. Inside living organisms, however, all syntheses and degradations of such molecules involve one enantiomorph alone.

Only the fact that chemistry is learned from the plane surfaces of paper and blackboard makes such selectivity seem strange. We tend to think of optical isomers as very much alike, but in fact they represent profound differences in *shape*; and, in the types of reaction upon which life depends, involving the ceaseless, intimate fitting together of molecular surfaces, shape is all-important. Organisms made the choice between optical antipodes long ago. To tamper with that choice now would be like trying to draw a left glove on a right hand.

How was this choice made initially? A start in either direction might be self perpetuating, but how was the original choice made? In the past I think discussions of this problem have been misdirected, in the sense that an attempt was made to propose ways in which inorganic devices might have produced populations of organic molecules of predominantly one configuration or the other which, on their later incorporation into living organisms, conferred their optical activity on the latter.

It is enormously more probable, of course, that all geochemical syntheses of organic molecules produced racemic mixtures. I think that organisms acquired optical activity, not as a gift from the inorganic world, but through processes of *selection* out of originally racemic mixtures. In what follows I shall try to outline the nature of such processes.

"Inorganic" Sources of Optical Activity

However, let me first consider several inorganic sources of optical activity for, though I believe them to be unimportant in a consideration of the origin of organisms, they have great intrinsic interest.

That right- and left-handedness are equally probable in disymmetric structures is both theoretically obvious and a matter of direct observation. To cause the products of an organic synthesis to display measurable optical activity demands the deliberate use of carefully chosen asymmetric physical conditions, or of optically active precursors, analytic reagents, or catalysts. Great ingenuity has been expended in attempting to show that asymmetry of physical conditions can sway a synthesis even slightly one way or the other.

For example, Cotton (1896) having demonstrated that optical isomers have

* The ideas discussed in this paper were first presented publicly at a symposium on biogenesis held at Brooklyn Polytechnic Institute, Brooklyn, N. Y., on May 7, 1955, and again at a public lecture at the Marine Biological Laboratory at Woods Hole, Falmouth, Mass., on July 1, 1955.

different coefficients of absorption for right and left circularly polarized light, it appeared that a racemic mixture of a disymmetric photosensitive substance, when exposed to circularly polarized light, might lose one of its components preferentially and so become optically active. After repeated failures, this effect was demonstrated by Werner Kuhn (Kuhn and Braun, 1929; Kuhn and Knopf, 1930; and Mitchell, 1930). Right circularly polarized light induced a trace of dextrorotation in α-brompropionic ethyl ester, and left polarized light induced a little levorotation. With α-azidopropionic acid dimethyl amide, a twentyfold larger result of the same kind was obtained, yet even here the largest rotation induced was only about 1°.*

Interesting as this phenomenon is, it could hardly play an important part in providing optical isomers for primitive organisms. Circularly polarized light occurs in nature (in moonlight, for example, and in the plane-polarized light from the sky that is reflected from the surface of the sea), and Byk (1904) suggested that the action of the earth's magnetic field should result in some degree of predominance of one form over the other. However, the excess of light circularly polarized in one direction is probably so small, the opportunities for its action are so restricted, and its effectiveness is so low that the net production of optical activity by this means under natural conditions hardly can be significant for our problem.

There has been considerable discussion of the possible induction of optical activity by the surfaces of asymmetric natural crystals. One thinks particularly of quartz, which constitutes about 15 per cent of the upper crust of the earth and, although composed of symmetric molecules of SiO_2, forms right and left crystals. It might be possible for a quartz surface to resolve optical isomers by differential adsorption and to promote asymmetric syntheses by oriented surface catalysis.

If such possibilities are to weigh in the balance, it is not enough to have right- and left-handed crystals; one or the other must predominate greatly. If this were not true, optical antipodes would be formed in equal numbers on neighboring right and left crystals, and the net effect over any considerable area would be racemic mixtures.

It seems to be true that, although right and left crystals of quartz occur equally in nature, one configuration may exceed the other in local deposits by a factor as great as 2:1 or, perhaps, even 3:1. V. M. Goldschmidt (1952) reported that he was told by "an experienced manufacturer of optical instruments" that the latter had found large, clear crystals of quartz to be right handed ten times as often as they were left handed. N. W. Pirie, in a footnote to the same paper, says he was unable to confirm this report with the

* It is difficult to understand what Oparin means in speaking of this result, acutally the first and less fruitful trial, as "complete success" (1938, p. 130). Oparin goes on to say, "These experiments furnished indubitable proof that asymmetric molecules can be produced without the aid of the living cell." It would have been better to say "produced outside of the living cell" and, perhaps, to add "with the aid of Werner Kuhn." This is otherwise like the oft-repeated statement that Wohler's synthesis of urea proved that living organisms are not needed to make organic compounds. What it showed, of course, is only that some living organisms, to wit, organic chemists, can make organic compounds externally as well as internally.

British firms that he consulted, though all agreed that right-handed crystals are a little more common.

Let us assume for the moment that limited areas of asymmetry in quartz might help produce locally asymmetric populations of organic molecules. One should expect to find adjoining areas in which the asymmetry went the other way. In the ocean, which is probably the most significant site for such events, mixing should help to eliminate local differences. I think that the outcome over any considerable area and interval of time should be virtually racemic. Furthermore, to my knowledge no one has yet demonstrated an optical resolution or an asymmetric synthesis on quartz. On the contrary, Seifert (1956) has found that when quartz orients the growth of amino acid crystals, D-amino acids are oriented equally effectively by right and left quartz. Altogether, there is little to encourage the suggestion that quartz has been the cause of any considerable asymmetry of organic molecules in nature.

How do local areas of asymmetry in crystals occur? They are thought to result from the phenomenon of seeding. Crystallization is precipitated in a supersaturated solution or melt by the presence of a seed crystal that serves as a nucleus for further growth. In a spontaneous crystallization, if the first microscopic seed crystal to appear were asymmetric, this might induce a further crystallization of the same orientation. In the case of such substances as quartz, in which only the arrangement of the molecules is disymmetric, the molecules themselves being symmetric, this phenomenon could organize an entire local crystallization in one orientation or the other.

This effect has been demonstrated experimentally with sodium chlorate, another symmetric substance that forms enantiomorphic crystals. Landolt (1896) precipitated aqueous solutions of sodium chlorate with alcohol, ground the crystals to a powder, and suspended this in alcohol-carbon disulphide to measure the optical rotation. When crystallization was rapid, little if any net rotation was observed; when crystallization was slow, marked rotations, both right and left, were observed. Landolt suggested that the fast crystallizations had begun from many centers simultaneously, and so were balanced, while the slow crystallizations frequently started from a single asymmetric center, and so were biased. Kipping and Pope (1898) allowed saturated solutions of sodium chlorate to crystallize spontaneously. Of 46 such experiments, there were only 2 cases in which the numbers of *d*- and *l*-crystals were equal. The percentage of *d*-crystals in each batch varied from 24.14 to 77.36, yet their weighted mean in all of the batches was 50.08 $\pm$ 0.11; that is, although the isolated crystallizations were almost always asymmetric, the total product from all of them was racemic. This is, of course, the expected result.

The same kind of experiment can be performed with disymmetric substances that form enantiomorphic crystals. Once again, it is possible to achieve a high degree of optical activity in isolated crystallizations performed in racemic mixtures. The process of crystallization itself resolves the mixture. In one instance to be cited it goes even further, and forces a racemization of the material still in solution into the orientation of the crystallizing isomer. These

are, therefore, remarkable instances of the spontaneous origin of optical activity.

All of the experiments of this kind of which I am aware, however, share a peculiar characteristic: the optical activity has almost always come out in the same direction.

Havinga (1954) has performed such experiments with methyl-ethyl-allyl-anilinium iodide. The molecular asymmetry in this instance derives from the attachment of four different groups to nitrogen, and the case is especially interesting since in organic solvents each enantiomorph is converted rapidly to the other, through the reaction:

Asymmetric Symmetric

Samples of the supersaturated racemic salt were sealed in glass vials and allowed to crystallize spontaneously. After standing at room temperature for several months, the tubes were opened. The crystals often were found to be highly active; the mother liquor was only slightly so, if at all. Apparently, much of the salt originally in the opposed configuration had racemized to the form that crystallized. The high degree of asymmetry achieved was all the more surprising because traditional methods for preparing this salt in an active state had been much less successful.

Havinga, however, speaks of having encountered a "serious difficulty." In a first series of fourteen trials, the crystals were dextrorotatory in twelve, never levorotatory.

Another series of experiments was performed with special precautions, which included careful filtration of the solutions through sintered glass. These solutions were reluctant to crystallize, and crystallization was finally induced by strong cooling. As Havinga notes, and as noted above in connection with Landolt's experiments, such a procedure tends to bring down both antipodes at once by forming multiple nuclei. Nevertheless, under these conditions Havinga also obtained a few instances of optical activity, this time both dextrorotatory and levorotatory. He concludes that this is probably the first instance in which the crystallization was "really" spontaneous; that is, in which some unknown contaminant may not have biased the result.

Havinga, however, is not the first worker to have had this kind of experience. He does not mention the experiments of Kipping and Pope (1898) and probably was unaware of them. These workers reported that, in ten spontaneous crystallizations of sodium ammonium tartrate from saturated solution, in every case the crystals were found to be strongly dextrorotatory, while the mother liquor was strongly levorotatory. The note in which these experiments were reported was written as part of a lively discussion that fol-

lowed a lecture to the British Association, in London, in the same year, by Japp (1898a), presenting a "vitalistic" view of the origin of optical activity in living organisms; and Japp, in a reply, (1898b) referred to still earlier experiments by Jungfleisch (1884) in which supersaturated solutions of racemic sodium ammonium tartrate were seeded simultaneously with a right and a left crystal, placed some distance apart in the crystallizing dish. Both optical isomers began to crystallize at once, but in the early stages of crystallization the crystals were always predominantly right-handed. Similarly, when the racemic salt was concentrated to such a degree that, on cooling, about half of it crystallized spontaneously, the mother liquor always was found to be slightly levorotatory, while the crystals apparently were slightly dextrorotatory.

Crystallization is a mysterious business at best mysterious because it is so sensitive to seeding and contamination that one frequently is not altogether sure why a preparation crystallizes or fails to do so. A vague mythology surrounds the process. Almost every laboratory has had the experience of struggling long and hard to crystallize something for the first time, only to find that thereafter it goes very easily, even though no attempt is made to seed. Perhaps this is evidence only that the workers are growing more skillful, but there is a widespread impression that after a first crystallization in any laboratory, microscopic crystals of this material are "in the air," that the entire laboratory is contaminated with them. One is told that the reason some English biochemists cultivate beards is to help their crystallizations the contents of a beard are thought capable of seeding anything!

When in such experiments as the above an investigator obtains 10 + results in 10 trials or 12 + results in 12 trials, one's first thought is that these are, after all, small numbers of experiments, of little weight statistically. The experiments are, however, impressive enough. If one thinks othewise, one may try tossing coins and coming up with 10 or 12 heads in a row. The chance of achieving 10 heads is 1:1024; of 12, 1:4096; that is, provided the chance of a head in a single toss is really 1:2.

A more acceptable view would be that such results are not due to chance, but to the effect of some unknown contaminant. In that case, if one were to grant that every laboratory provides a bias, right or left, for whatever crystallizations it undertakes, the chance of three laboratories coming out the same way independently is 1:8. Therefore this is not very improbable.

However, this seems to me a strange way in which to leave the matter. I think that a few more experiments in this area would not be amiss, for perhaps we are assuming too much too lightly. One would like to know more about the hypothetical contaminant. What is the nature of a contaminant that could determine an asymmetric crystallization? Why, even in one laboratory, should it always determine in the same direction? Indeed, in one laboratory (Kipping and Pope, 1898) such a symmetrical substance as sodium chlorate yielded equal numbers of right- and left-handed crystallizations; while, with such a disymmetric substance as sodium ammonium tartrate, only the dextrorotatory isomer crystallized. Therefore, the bias appears to be introduced at the level of molecular disymmetry; it does not appear to predispose the crystallization molecules in one orientation rather than the other.

Consequently we must consider the presence of contaminants, themselves highly asymmetric, all of the same configuation in one laboratory, and perhaps all of the same configuration in many laboratories.* What can this mean? Indeed, if there is any reality in this picture, I can think of only one explanation, and that is that the contamination comes from living organisms, perhaps from the experimenters themselves. If this is so, it is a curious inversion from what was first intended. These experiments had set out, in part, to support the thesis that optical activity, once it had arisen spontaneously in the inorganic world, had been impressed subsequently upon organisms. They may demonstrate just the converse; that is, that in a world contaminated with life, disymmetric substances may no longer be free to crystallize at random, but are biased toward the configurations that living organisms have already chosen.

And what if the bias is not caused by contamination? Certainly the Jungfleisch experiment, in which solutions were deliberately seeded with a right and a left crystal, does not lend itself readily to this type of explanation. Behind our conviction that both enantiomorphs should be equally apt to crystallize is the tacit assumption that all conditions that surround the process are symmetric, or at least not sufficiently unsymmetric to matter. Perhaps the attendant physical conditions need more attention than has been given them thus far.

I hope I have not made too much of this issue. I am as reluctant as the reader to quarrel with a concept as well founded as the one that holds that the spontaneous crystallization of L- and D-isomers should be equally probable. The trouble is that the only data that I have found indicate the contrary, undoubtedly for very good reasons that leave the fundamental concept intact. I should like to know these reasons.

To return to our primary problem: the origin of optical activity in living organisms. I do not think that the phenomenon of spontaneous crystallization can help significantly with this, as its conditions are much too particular:

(1) One condition is supersaturation, since resolution occurs only through crystallization.

(2) In general, in spite of the experimental aberration discussed above, one should expect the area of optical activity developed by this means to be highly localized, and to be balanced by neighboring areas of crystallization opposite in sign, and the product as a whole to be virtually racemic.

(3) A final condition is by far the most discouraging: the two enantiomorphs must form separate crystals, rather than crystallize together. Pasteur's famous separation of D- and L-tartrate crystals by inspection has created the impression that the formation of such enantiomorphic crystals is a common

* It is not essential, however, that the contaminant that is responsible for seeding should contain an asymmetric carbon atom (Ostromisslensky, 1908). Supersaturated solutions of DL compounds, on seeding with a crystal isomorphous with the D or L form, may precipitate that form exclusively. For this the seeding substance must form enantiomorphic crystals, though these need not necessarily be composed of disymmetric molecules. Thus, optically active asparagine (D or L) can be precipitated from supersaturated solutions of DL asparagine by seeding with single crystals of glycine, which apparently are enantiomorphic, though not visibly so.

phenomenon. In fact, it is rare; few substances are known to have this property.

Indeed, all the inorganic sources of optical activity share the same disabilities: very restricted conditions, a very limited field of operation, poor yields, and the overwhelming tendency to result in only local and temporary asymmetry in what is otherwise a racemic continuum. For the origin of optical activity in living organisms, I think one must look elsewhere.

In what follows I shall try to develop the thesis that optical activity appeared as a consequence of intrinsic structural demands of key molecules of which organisms were eventually composed, through the selection of optical isomers from racemic mixtures. I think that recent work on the structures of proteins and nucleic acids has begun to provide a basis for this type of hypothesis.

Optical Activity by Selection: Polypeptides and Proteins

Pauling and Corey (1951a) have shown that the most prevalent type of physical configuration assumed by proteins is the so-called α-helix (FIGURE 1). This is a very tightly wound structure that contains 3.6 amino acids per turn, in which every amide group (—CONH—) is hydrogen bonded to amide groups that are three amino acid residues removed in either direction along the chain. The structure is very much more condensed than indicated by such a diagram as FIGURE 1, which shows only the positions of the atomic centers, not their radii. The α-helix is a rod rather than a tube; inside it the atoms come close to being in van der Waals contact.

It is important to realize that this structure forms spontaneously. For proteins, or portions of them, which can assume this configuration, under usual conditions it represents the most stable state: that at which the free energy is at a minimum. In a reversible denaturation a protein is exposed to special conditions that cause the helix to uncoil; on the return to "native" conditions, the protein spontaneously reassumes the helical configuration.

Not all proteins form α-helices, and those that do may not be entirely in this configuration. Various incidents of polypeptide structure (internal rings, proline residues) may interrupt or distort the helical structure. It is possible, by measuring the optical rotation, to estimate how much of a protein or polypeptide is in this form, since the helix itself contributes a large optical rotation ($[\alpha]_D = 48°$) (Blout, Doty, and Yang, 1957). It is estimated that the helical configuration accounts for 10 to 50 per cent of the structures of many native proteins in aqueous solution (Doty, 1956).

Recent studies show that synthetic amino acid polymers in certain solvents assume the α-helical configuration spontaneously (Pauling and Corey, 1951b). Thus, poly-γ-benzyl glutamic acid is helical in acid solution, in which the side-chain-COOH groups are uncharged, but uncoils to a random configuration when these are ionized in alkaline solution, because of the mutual repulsion of the negative charges along the polypeptide chain. These changes are freely reversible (Blout and Idelson, 1956a).

Consequently, the α-helix appears to represent a particularly stable physical configuration which proteins, polypeptides, and synthetic amino acid polymers

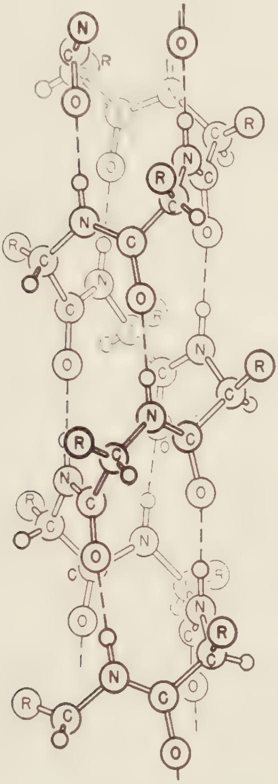

FIGURE 1. Diagram of a section of an α-helix. The R-groups of the amino acid residues are attached as in the L configuration, and the right handed helix that is characteristic of this configuration is shown. The actual structure is much more condensed than appears in this diagram, which shows only the positions of the atoms, not their radii. This figure is reproduced by the courtesy of J. T. Edsall, and is taken from an unpublished figure by L. Pauling and R. B. Corey.

assume spontaneously when they can, and to which they return spontaneously after interruption.

Can a polypeptide composed of a mixture of L- and D-amino acids form the α-helix? The first answer to this question is that it can. As FIGURE 1 shows, the R-groups of the amino acids do not take part directly in the α-helix, but project from it. A carefully constructed space model of the α-helix shows that it possibly could be wound right- or left-handed, with L- or D-amino acids or with mixtures of L- and D-amino acids.* It is also true, however, that the mixed structure involves possibilities of steric hindrance between adjacent R-groups that do not exist when a single optical configuration is employed.

* I am grateful to Barbara Low, to whom I brought this problem while she was at Harvard Medical School, Boston, Mass., and who was helpful in clarifying it with the aid of her carefully constructed model of the α-helix.

The hindrances involve principally the groups attached to the β-carbon atoms of the side chains. For this reason a polypeptide chain composed largely of glycine residues might encounter very little hindrance among its other amino acids, even if their configurations were mixed. However, proteins in which the amino acids were more widely distributed would encounter difficulties. Certainly, a few D-amino acids might be introduced into a helix made primarily of L-amino acids (or the other way around) without serious hindrance, but the more widely this occurred the greater instability should result, and anything approaching a random assortment of L- and D-residues might make the α-helix impossible.

If the formation of α-helices plays an integral part in the making and maintenance of proteins, and if this in turn is enhanced by the employment of a single configuration of amino acids, that in itself should provide sufficient basis for the selection of one configuration out of mixtures of enantiomorphs.

When I became interested in this solution of the problem several years ago, I asked my colleagues P. Doty and E. R. Blout, who were studying the properties of synthetic amino acid polymers, whether it might be possible to demonstrate experimentally the selection of single optical isomers out of a racemic mixture in the course of synthesis of such polymers. I was told that experiments that would bear upon this question had been planned, and a number of them have been performed since that time.

I should like to summarize briefly the present results of these studies. A helix composed of L-amino acids contributes a large dextrorotation to the optical activity, and apparently has the absolute configuration of a right-handed screw. Conversely, D-amino acids form a left-handed helix, and contribute a levorotation (Blout, Doty, and Yang, 1957). When the polymerization of an amino acid anhydride is initiated, the polymer propagates slowly until it reaches a length of about eight residues. At this length, at which it apparently first forms a stable helix, the rate of propagation suddenly increases many times and remains rapid thereafter. That is, the α-helix appears to grow very much more rapidly than an unorganized polypeptide chain (Doty and Lundberg, 1956).

An L-polymer can be used to initiate the polymerization of L-, D-, and DL-amino acid anhydrides. Such experiments have been performed with γ-benzyl glutamic acid anhydride (Doty and Lundberg, 1956). An L-polymer growing in L-anhydride yields a rapid and continuous increase in dextrorotation caused by the growing right handed helix. The L-polymer in DL-anhydride grows more slowly, but it still increases gradually in dextrorotation, not primarily because of preferential incorporation of the L-anhydride, but because the right-handed helix of the initiating polymer continues to grow as a right-handed helix in spite of the incorporation of both D- and L-glutamate. The D-anhydride yielded a curious result. The dextrorotation increased for a short time, then reversed, and thereafter grew in the direction of levorotation. The initiating right-handed helix had propagated as a right-handed helix while incorporating the first 3-4 D-residues; then it reversed itself and grew thereafter as the left-handed helix characteristic of the D-polymer.

What interests us most is the comparison of such polymerizations and their

products when they involve DL mixtures of amino acids as compared with the single D- or L-configurations. Such studies already have demonstrated the following properties:

(1) The DL-polymer propagates much more slowly than the L- or D-forms. In recent experiments with γ-benzyl-N-carboxy glutamate anhydride, the DL mixture polymerized at only one twentieth the rate of either the D- or L-anhydride (Blout and Idelson, 1956b). The effect is therefore much greater than if the growing polymer merely selected only one enantiomorph out of the racemic mixture, for that should cut the rate only to 1:2. Apparently there is no such rigorous selection; it seems rather to be the lowered tendency of the DL-polymer to assume the helical configuration that inhibits its rate of propagation.

(2) The DL-polymer is considerably shorter in length than D- or L-polymers that are formed under the same conditions. Thus, in the case just cited, the average weight of the DL-polymer was only one fourth that of the L- or D-forms (Blout and Idelson, 1956b).

(3) The DL-polymer is much less stable in configuration than the L- or D-forms. It forms α-helices under favorable conditions; but when subjected to unfavorable conditions, the DL-helices "melt out" (the equivalent of denaturation in a protein) long before helices composed of a single configuration of amino acids (Blout, Doty, and Yang, 1957). That is, the DL-helices are "weak" even in favorable solvents. In aqueous solutions, in which water tends to form hydrogen bonds with the amide groups and thus to compete with their formation of such bonds with one another, the α-helix has a lowered stability at best. In such solutions a DL-polypeptide probably could not form an α-helix at all.

In these experiments the polymerization of amino acid anhydrides does not select single optical isomers efficiently out of racemic mixtures, although Doty tells me that evidence of some degree of selection has begun to emerge. Perhaps such evidence might be more pronounced if the experiments were performed with β-benzyl rather than γ-benzyl amino acid anhydrides since, as already noted, it is principally the groups attached to the β-carbon atom that should hinder the formation of α-helices.

It must be added, however, that this type of system is not well adapted to selecting L- or D-amino acids out of DL-mixtures, for the propagation reaction is irreversible; a unit added to the polymer chain is attached permanently. Consequently, there is no opportunity for the polymer, by repeated interchanges of amino acid residues with the solution, gradually to achieve its most stable composition. If one could grow such polymers in a reversible system in which synthesis was partly balanced by hydrolysis, the opportunity for selection would be greatly improved.

To summarize this argument: the mixture of D- and L-amino acids in the composition of polypeptides, by hindering the formation of α-helices, has been shown to result in greatly lowered rates of growth, decreased structural stability, and smaller size. Polypeptides composed of single optical configurations of amino acids are at an enormous advantage in all these respects. I should suppose that these relationships already provide an adequate basis for a high degree of selection of single optical isomers from racemic mixtures of amino acids

under any conditions that might be imagined to have governed the "spontaneous" formation of primitive polypeptides and proteins.

As such molecules aggregated with others to form structures of still higher order, organized aggregates and, eventually, living organisms, the importance of forming helices and the consequent pressure to select single optical isomers, must have grown steadily. It is the helical configuration that gives a protein its specific shape; the alternative is a random skein of no shape at all. Upon their specific shape depends the solubility of proteins, their capacity to crystallize and to enter into other types of regular orientation with respect to other molecules, and their specific chemical reactivities. Hence, all these properties decline or are lost on denaturation; that is, on the loss of the helical configuration. It seems to me that the tendency to segregate single optical isomers of the amino acids, which had begun because it was advantageous in the formation of simple polypeptides, must have become more and more rigorous as proteins became larger and more complex, as they joined with other types of molecule to form more and more complex and highly organized aggregates,

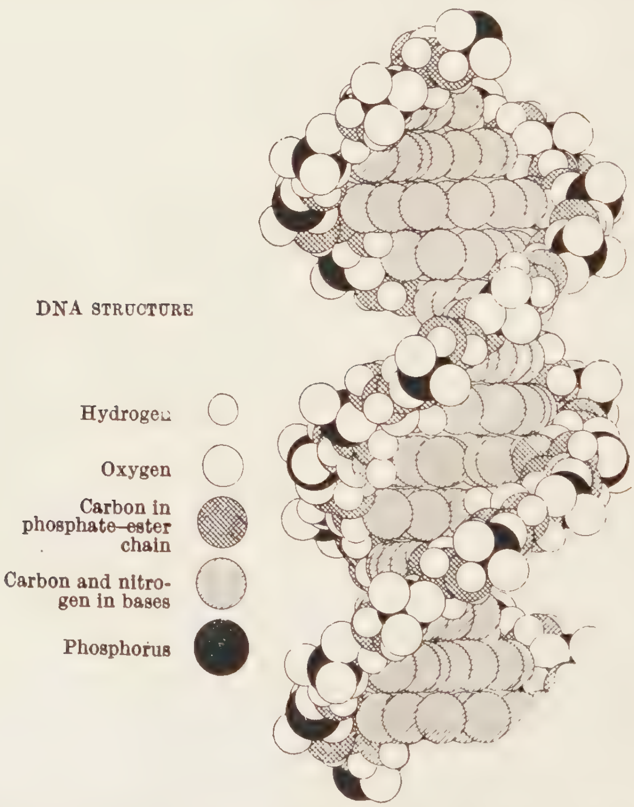

FIGURE 2. Space model of the double helix of DNA, according to Watson and Crick (1953). The envelope of the helices is formed by a backbone structure of alternating residues of D-deoxyribose and phosphoric acid. The nitrogenous bases form the horizontal rungs that run between the helices. (From Feughelman *et al.*, 1955.)

and as they eventually entered into the composition of living cells. What had begun as a relative advantage must have ended as an absolute requirement.

Optical Activity by Selection: Nucleic Acids

A much stronger, because simpler, case of the same kind can be made for the nucleic acids. Both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) occur in the form of regular helices. Let us first consider the structure of DNA, for which a reasonably satisfactory model proposed by Watson and Crick (1953) is shown in FIGURE 2.

The entire geometry of this structure depends upon the arrangement of groups about asymmetric carbon atoms of the deoxypentose (FIGURE 3). The component nucleotides are all β-glycosides, which therefore always involve the same orientation of the pyrimidines and purines toward C_1 of the pentose. The phosphoric acid linkages between successive nucleotides run from C_3 of one pentose to C_5 of the next (Todd, 1954). C_3 is asymmetric, while the orientation

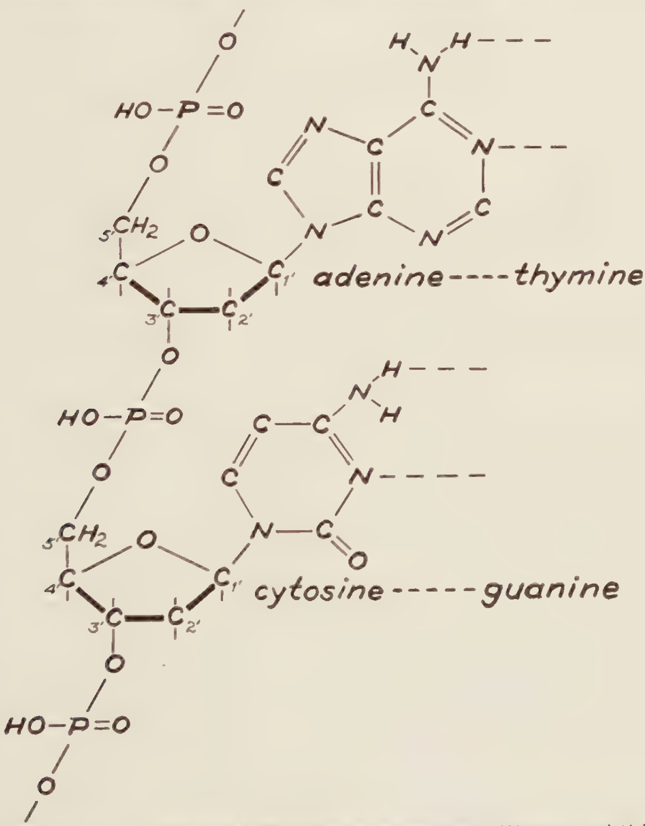

FIGURE 3. Diagram of a section of a DNA helix, according to Watson and Crick (1953). The structure depends upon specific orientation of the groups attached to asymmetric carbon atoms 1′, 3′, and 4′ of D deoxyribose. The broken lines that lead off to the right from the nitrogenous bases represent pairs of hydrogen bonds that run from the adenine and cytosine in this helix to thymine and guanine in the complementary helix to which it is attached.

of C_5 is determined by the fact that we are dealing throughout with D-deoxyribose; that is, with a specific orientation of C_5 with respect to the asymmetric C_4. All of this leads in the Watson-Crick model to the formation of a right-handed helix; and two such helices can be united so that a given pyrimidine in one always forms a pair of hydrogen bonds with a given purine in the other. The pairs of bases thus form steps on the helical staircase, as shown in FIGURE 2.

Consequently, specific choices involve the arrangement of groups about the asymmetric carbons 1′, 3′ and 4′ of the deoxypentose. The opposite choice in each case might have led to a stable, though different, structure, but a single choice one way or the other is essential. If the configurations about C_1 were mixed (that is, a mixture of α- and β-pentosides), the nitrogenous bases could no longer meet to form a paired structure. If the configurations about C_3 or C_4 were mixed, no helix could be formed.

RNA presents essentially the same problems, although here we do not yet possess so detailed a structural model. Again, the X-ray diffraction data indicate a helical configuration and a regular structure, very likely at least two-stranded, as Warner's paper in this monograph suggests. As in DNA, this structure must derive from the specific arrangements of phosphoric acid and bases about the pentose carbon atoms, which are the same in RNA as in DNA (Rich and Watson, 1954).

The polynucleotide phosphorylase discovered by Grunberg-Manago and Ochoa synthesizes *in vitro* polynucleotides of large molecular weight that resemble RNA closely in structure, size, and behavior toward various enzymes (Grunberg-Manago, Ortiz, and Ochoa, 1955). X-ray diffraction studies show that such synthetic polymers also assume the same helical configuration as does natural RNA. The helical structure therefore must represent the most stable configuration for this type of molecule, just as the α-helix does for polypeptides.

I think it eventually may be possible to demonstrate with some synthetic system the selection of single optical configurations from mixtures of nucleotides in which the pentoses are epimeric to those found in the natural nucleotides as regards carbon atoms 1′, 3′ and 4′.* If it is true, as we suspect, that the formation of helices contributes stability and may be otherwise advantageous to the formation of polynucleotides, then this process should effectively select the configurations of nucleotides that promote helix formation out of any mixtures of optical isomers that may be offered. If the synthetic system also were reversible, any disadvantageous sequences formed initially could be corrected later by exchanges between the forming polymer and the solution, until the most stable configuration had been achieved.

Right or Left?

If it be granted that optical isomers were selected as described above, this still tells us nothing of why one isomer rather than the other was chosen. Why

* Of course, one could not use polynucleotide phosphorylase or any other enzyme for such a demonstration, since all enzymes, being proteins, are themselves asymmetric and can impress asymmetry on the reactions that they catalyze.

are living organisms composed of L- rather than of D-amino acids? Or of D- rather than of L-ribosides?

I once had the pleasure of discussing this matter with Albert Einstein. He had asked my opinion, and then said: "You know, I used to wonder how it comes about that the electron is negative. Negative– positive– these are perfectly symmetric in physics. There is no reason whatever to prefer one to the other. Then why is the electron negative? I thought about this a long time, and at last all I could think was 'It won in the fight!' " I said, "That's just what I think of the L-amino acids. They won in the fight!"

In other words, if the external forces at work are indeed as symmetric as we suppose, we must assume that the original choices between optical isomers were arbitrary. At just which points such choices were made is problematical. One might well imagine a time at which there existed proteins made of either L- or D-amino acids, although for the reasons I have given I would suppose that each such molecule tended to contain one configuration alone. Similarly, there might have been nucleic acids made with L-ribose and others made with D-ribose. It is much less likely that both groups were mixed in single organisms, even at a very primitive level, for an organism could hardly survive for any length of time if it had ceaselessly to sort out the configurations of its unit molecules in making structures of higher order, or in carrying out the chains of connected reactions upon which its metabolism depended.

Perhaps, however, there were at one time two populations of organisms, L- and D-. I am speaking loosely here of configurational relationships that hold among large series of molecules, not of directions of optical rotation, and I am also greatly oversimplifying the possibilities, which of course could include a variety of intergrades. For a time, two such enantiomorphic populations might survive together. However, as organisms began to live on other organisms, to pass material from one to another through long food chains, and to live in and on the products of other organisms, it would become highly advantageous not only for each individual organism, but for all of them collectively, to utilize single configurational series of molecules. Anything else would create endless difficulties. We see a particular instance of such difficulties in antibiotics such as gramicidin and tyrocidin that may owe their antibiotic effect, in part, to their high content of the unnatural D-configuration of amino acids.*

Therefore, I should suppose that the ordinary forces of natural selection would quickly force the choice of one or the other enantiomorph, eventually, for all life on this planet. Very early in evolution certain organisms (or perhaps precursors of organisms) which, for other reasons were superior to their enantiomorphic neighbors, won out in the struggle for existence—the "fight" and this decided the matter for all time.

This conception has a curious consequence. If we are correct in believing that life arose through wholly natural processes, then, given enough time, it should appear wherever conditions in the universe permit. The conditions

* Katchalski *et al.* (1955) have shown, however, that in a number of antibiotically active amino acid polymers related to gramicidin S, which itself contains D-phenylalanine, the replacement of D- by L-phenylalanine has little effect on the antibiotic activity.

are reasonably restricted; they cannot depart very widely from those found on the earth.

It is estimated conservatively that about one hundred thousand planets such as the earth exist in our galaxy; since about one hundred million galaxies are within range of our most powerful telescopes, there may be several million million such planets in the observed universe (Hoyle, 1950).* It is difficult to avoid the conclusion that life exists on many of them life as we know it; and that optical activity, since it is advantageous, is as characteristic of life elsewhere as here.

If the choice of optical isomers is as arbitrary as proposed one should expect that a survey of life throughout the universe would reveal approximately equal numbers of planetary populations in which the choice of metabolically connected series of disymmetric molecules came out L- or D-; roughly equal numbers in which life is based upon L- and upon D-amino acids and, similarly, for the other molecules.

Indeed, now that we have begun to deal in anti-universes, perhaps it will become possible to pursue Einstein's question a little further. If one could sample all of the universes in the multiverse, perhaps one would find roughly equal numbers in which the electrons are positive and negative. Or does that assume too much symmetry?

Conclusion

In this discussion I have attempted not to summarize but to make a beginning, with a particular type of hypothesis concerning the origin of optical activity in organisms. The essential idea is that from unit molecules provided by geochemical processes in racemic mixtures, single optical isomers were selected in the process of composing structures of higher order: polypeptides and proteins from amino acids, nucleic acids from nucleotides, and so on. The basis of the selection was that the structures of higher order gained stability and other advantages by assuming physical configurations demanding that only one optical configuration be employed. The final choice of one of the two optical isomers in each instance is assumed to have been arbitrary.

I have developed this line of argument for two particular instances; to wit, proteins and nucleic acids. It probably could as well have been constructed around a wide variety of other types of compound. The structures and stability of the polysaccharides, particularly such structural polysaccharides as the celluloses, demand specific choices among the epimers of glucose. The natural lecithins are all dextrorotatory. One of their most striking properties in water is to form beautifully oriented, double-layered films; could they do so as well if the optical isomers were mixed?

It is not yet clear how many such choices must be made. The molecules that compose organisms are interconnected elaborately by long and complex metabolic chains of reactions. When the optical configuration of a key member of such a series is determined, this determines many others. It would be

* Hoyle (1955) has since greatly increased this estimate, indeed by about 100,000 times. However, even if the first, lower estimate were already too great by a factor of a million, that would not change our argument.

interesting to discover how many such interdependent series of asymmetric molecules the organism contains, and what may have been the mechanism of selection in each instance.

Finally, I think it should be said that the type of mechanism here proposed for introducing optical activity into the structures of proteins, nucleic acids, and other molecules, and through them into living organisms, is in good accord with our general conception of the nature of the evolutionary process. Evolution advances, not by a priori design, but by the selection of what works best out of whatever choices offer. We are the products of editing, rather than of authorship. The process I have pictured here, in which single optical configurations are chosen out of racemic mixtures because they work best in forming structures of higher order, agrees well with this general principle. It is an example of natural selection on the molecular level.

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SOME ASSUMPTIONS UNDERLYING DISCUSSION ON THE ORIGINS OF LIFE

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A few discussions can reasonably be expected to end with a definite conclusion. They are organized to consider where or how something happened, or what something was made of, and we can see at the start that there is a general line of research that should produce the answers. Discussions about the origins of life are not like this nowadays. It may be that before the seventeenth century the argument seemed reasonable that, if frogs and other animals could arise from mud now, they must have arisen in that way in the beginning. However, the issue was not considered very important. We now have little expectation of being able to conclude a discussion with the statement "this is how life did arise"; the best we can hope for is "this is one of the ways in which life could have arisen." All that we can do is to compare probabilities and, if we are to do that usefully, it is essential to keep all of the possibilities in mind. It is clearly impossible to state all the possibilities in one brief paper. Many of these need not be mentioned here because they figure in discussion so often that no one could overlook them; others are mentioned to show that they have been borne in mind, and the remainder have been selected, not because I regard them as being intrinsically highly probable, but because they are in danger of being overlooked entirely.

First among the possibilities to be mentioned, but to be discussed only briefly, is that life had no origin. This is one of the ways in which what is sometimes called the "perfect cosmological principle" could operate. According to that principle the universe has had, apart from local fluctuations, the same appearance at every epoch. If so, there must have been life in it always. This could arise constantly of necessity by one of the processes that we shall discuss, or it could drift around the universe as what Haldane (1954b) calls astroplankton; vital units ready to start the cycle of evolution when conditions are favorable.

If the universe had a beginning, so clearly had life. Our decision about how far back in the history of the universe this beginning occurred depends on the wholly arbitrary criteria that we decide to use to distinguish the living from the nonliving state. If nothing can be called alive unless liquid water is a prominent component of it, then life can start only on a cool planet, and so on. However, if we try to define life operationally, there is no need for this restriction. I argued twenty years ago (Pirie, 1937) that a rigid operational definition is not possible. This seems now generally to be accepted. Nevertheless, those who work with viruses are still asked whether their experimental material is alive or not or, more often, are told dogmatically that it is or is not. At most, all we can say is that we prefer not to call a system alive unless it has a certain proportion of the qualities in a given list. It is possible, in theory, for these qualities to be manifested in systems that consist predominantly of gas or fused

rock, as in the systems proposed by Preyer (1880) and by Pflüger (1875). Esthetically we tend to reject so wide an extension of the living domain. A gaseous system would not even satisfy Hopkins' (1913) minimum requirement that for life there should be a "dynamic equilibrium in a polyphasic system."* Still, the possibility remains that such systems were the ancestors of the systems we now know, even if such an origin is not very probable.

The only justification for paying any attention at all to ideas about interstellar or incandescent life lies in the assumed improbability of its spontaneous appearance under present conditions. The ancients did not make this assumption. It was forced on us, first by the success that Redi and Spallanzani had in defining experimental conditions that excluded the grosser forms of life, then by the commercial success of Appert and Bryan Donkin, who excluded the smaller forms and, finally, by Pasteur and Tyndall, who put the matter on a scientific basis. We all now agree that the appearance of life in any vessel under observation is so improbable that when it is claimed, as by Bastian and Pouchet, we put it down dogmatically to the result of faulty technique. In this we are probably correct, but our attitude tends to impede further experimentation. In the absence of experiment, can we invoke theory to put a value on this improbability?

This has been attempted in several different ways. Guye (1942) assumed that the problem was tantamount to considering the probability of the spontaneous appearance of a protein molecule. He made a series of dubious assumptions, wove his fabric together with some physicochemical fallacies, and concluded that the universe had not existed long enough to allow one protein molecule to appear spontaneously. Other similar attempts have been made. There are two basic fallacies in this type of approach: (1) it assumes that there is only one way in which a certain state of affairs, such as life, can exist; and (2) it assumes that the probability of a process can be calculated although its mechanism is unknown.

Let us consider the latter objection first. No one would try to calculate the probability of catching a fish of known weight in a pond of known volume without knowing something of the habits of the fish and the size of the holes in the net; nor would we calculate the accident rate from the number of vehicles on a given area of road without considering traffic conventions and the extent to which they are obeyed. Chemical reactions at catalytic surfaces are similar. The rate at which a certain polymer will be formed depends on the rate of adsorption, the rate of the reaction after adsorption, the stability of the first links before polymerization is complete, and so on. As a rule, these are not known, and the rate must be determined experimentally. It is also very relevant to this aspect of the discussion that the type of polymer built up in a given reaction mixture depends on the catalyst used. This has long been known in gas reactions; now Natta *et al.* (1955) find that, by suitable choice of catalysts, a series of nonrandom polymers can be made with properties that are sharply

* Hopkins did not claim to be the first to emphasize the importance of phase boundaries for living organisms. It is interesting to note that he had a forerunner in Jean Rey (1630) who, considering why lead got heavier when calcined, concluded that life had nothing to do with the matter because lead, as "a homogeneous body without distinction of parts," could not be alive.

distinguished from those of the products of ordinary polymerization. If specificity and regular arrangement among the component parts of a macromolecule can be introduced in this way, there is no reason to assume that all was random under probiotic conditions.

By studying present-day living organisms we see some of the ways in which they can function. This does not tell us that these are all the ways in which they have ever operated; many types of metabolism may have died out. Still less does it tell us that these are all the ways in which they could have worked. Given the chemical components of some present-day organisms (proteins, fats, polysaccharides, nucleic acids, and so on) we cannot construct a viable organism. The fact that no one has made (or, so far as I know, has even tried to make) a para-organism by the use of colloids such as silicates, polyphosphates, and cyanides, chelating agents such as ethylenediamine tetraacetate and the metaphosphates, and oxygen carriers such as rubrene, does not therefore rule out the possibility that there could be such an organism. When we predict a probability, it is the probability of something foreseen; it is obviously possible to predict that certain arrangements could not work, but it is not possible to assert that we have foreseen all of the arrangements and chemical patterns that could work. Science is only retrospectively logical. Thus, when a hand of cards is dealt, it may be certain that there will be five cards in it; the 1:1,000,000 odds are against any predetermined set of five. Unless we know how many of the possible hands could be "effective" for our purpose, there is no basis for calculating the odds on receiving an "effective" hand. Similarly, we cannot calculate how often a molecule that could have been the vehicle for life could have arisen in any given time or conditions.

Haldane (1952), as so often before, has introduced some clarity of thinking into the subject. He gives reasons for believing that a simple organism such as a bacterial virus contains about 100 bits of negative entropy or information, and that this is about the amount that would arise spontaneously in 10^9 years in the volume of the primitive ocean. Viruses are probably not a step in the route to genuine, free-living organisms, but probably are either degenerate organisms or the consequence of metabolic blunders in an organism (Pirie, 1952 and 1955). Their relevance here is that they probably contain as many bits as does an eobiont, with too few of some sorts and too many of others. Haldane's argument suggests that many quite complicated systems could have appeared complete in the time available. There is therefore no necessity to look for some extra terrestrial source or evocative agency for life. Even if life does pervade space, there is no need to assume that our forms came from space. This is not a question that will be settled immediately by astronautics, because only a very rudimentary form of astroplankton would be expected to survive in space. Its subsequent development, in the different environments of different planets, probably would be so different that it will be difficult if not impossible to decide whether such similarities as may be found are the results of adaptive convergence or common ancestry. No one has suggested a valid means for telling whether the organisms we already know had one origin or many. Whatever their relative importance in human affairs, nurture probably has dominated nature in a contest that has lasted 2,000,000,000 years.

If, therefore, as seems probable, polymers and other substrates for life had accumulated in certain regions of probiotic Earth, waiting for chance to organize them, do we know to what kind of substance this chance happened, or to what kind of substance it could have happened? I maintain that we do not, and that the obsession with proteins and phosphoric esters, which is such a feature of the literature of this subject, may be based on an illusion. Admittedly, nothing that anyone wishes to call alive has been shown to be free from protein; but the search has been neither complete nor deliberate. The demonstration that all present-day forms of life depend on proteins would prove no more than that proteins are the most efficient way of living, and that they have superseded any others that may at one time have existed. Similarly, paper has superseded more primitive materials for writing, and money the more primitive means of exchange.

There is at present no reason to believe that proteins have any merit in living systems other than their colloidal properties and the possibilities for specificity that they offer. This potentiality, if we assume that every difference is biologically significant, is vastly in excess of anything that organisms can use. A peptide chain that contains 120 amino acids of 20 types could have more than 10^{130} arrangements, and the possibilities of configurational difference would be increased still more by folding and cross-connection. An organism weighing 70 kg. could contain only about 10^{23} molecules of such a protein. Most of the 2,000,000 species that may now exist are smaller than this, and it is unlikely that they have been preceded by 10^{100} more primitive species. There is, therefore, a fantastic superfluity of specificity available; every protein molecule that has ever existed on the earth could have been different. It is not, therefore, surprising that Fox and Homeyer (1955) find that even in their compositions, to say nothing of their configurations, proteins are not as variable as they could be. This is compatible with, though clearly not evidence for, the idea that protein complexity is a domain that organisms are only beginning to exploit. Haldane (1954a) makes a similar point. He accepts the common opinion that there is a trend toward biochemical simplification among the higher organisms, but he shows that this trend is obvious only when attention is confined to small molecules. The distribution of large molecules shows the reverse trend for the number of proteins and other antigens produced by each member of a species appear to increase as evolution proceeds. Haldane argues that this could broaden the range of action of enzymes and other active proteins usefully, and thus could open up possibilities of adaptation that are valuable enough to compensate for the difficulties that incompatibility introduces when a species reproduces sexually.

Consequently, if the full potentialities of proteins as biological agents are still used so inadequately, there seems to be no reason to think that life could not have originated among colloids with fewer potentialities if the smaller number were used more fully. Recently the idea that proteins have a fundamental role has gained strong support from the demonstration (Miller, 1953) that amino acids are among the products made when electrical discharges pass through certain gas mixtures. This is an observation of great importance because it adds another group of substances to those that it is reasonable to postu-

late as components of a probiotic environment. However, it is equally important to combat the assumption that the observation demonstrates either that the primitive atmosphere was a gas mixture similar to that used or that amino acids were components of the original organisms. These remain possibilities and nothing more. Even if the experiments are extended so that proteinlike materials are synthesized *in vitro* by similar processes, there will still be no proof—only a stronger probability.

Proof or disproof of the original significance of proteins will be very difficult to obtain. The anatomy of many early fossils makes it reasonable to assume that in many ways these organisms functioned in the same manner as those of the present day and, consequently, used protein. That carries us back 500,000,000 years at most—only one third of the way to the time when living processes may have started. There are two justifications for remaining skeptical in face of the general assumption that proteins must have been involved at the beginning. One of these is that for as long as this is assumed, insufficient effort will be put into the attempt to find ways to obtain genuine evidence. The other is summed up with pleasant irony by Hilaire Belloc:

> "But Scientists, who ought to know,
> Assure us that (it) must be so. . .
> Oh! let us never, never doubt
> What nobody is sure about."

The conclusion to be drawn from this is that even if such substances as proteins were essential at the beginning, the spontaneous appearance of organisms is reasonably probable and, if a more extensive group of substances could have been the vehicle for the operation, it becomes still more probable. This was the opinion of Darwin, Huxley, Tyndall, and others. They looked on the process as so improbable as to be unlikely in any vessel under observation, but they thought that, if enough space and time were available, it could happen and even happen frequently.

This type of argument could be pursued through the various types of substance now most intimately associated with living (sugars, purines, and so on) to see whether we must postulate an essential role for any of them at the beginning. That would be tedious and we may move to the other extreme and consider the elements. To the group of generally accepted bioelements—C, Ca, Cl, Cu, Fe, H, I, K, Mg, N, Na, O, P, S, Si, and Zn—we must now add B, Co, Mo, Mn, and V, while Al, As, Ba, Br, Cr, F, Ni, Se, and Sr, appear as possibles that we may soon be forced to accept. This composes one third of the naturally occurring elements, and it would be foolhardy to contend that the list would then be complete. The properties and sites of occurrence of Ga, Ge, Th, and Ti suggest that these also may have, or may have had, biological roles. If organisms now have this catholic approach to chemistry, by what canon would we decide which reactions were significant for the original organisms?

It is often argued that the most common environment is the one in which life most probably originated. There are two objections to this. First, it

amounts to the assumption that any environment and material would be equally suitable; the probability would then depend simply on the scale of the environment. When put in this way, the argument is generally seen not to be attractive. Esthetically, people cling to the idea that biopoesis was an activity of rather special regions and substances. Furthermore, it is commonly seen that evolutionary advances are now characteristic of atypical habitats. Second, the assumption cannot logically be made by one who believes in the primacy of proteins. Silica, carbonates, phosphates, and aluminates would be very much more probable vehicles if quantity were the important factor. Indeed, a case can be made for the original importance of these compounds because they play a prominent role in many ancient species.

If the origins of life took place in regions that were, for various reasons, distinctive, it is profitable to consider what the nature of the favorable peculiarity might have been. First, as nearly everyone has agreed, is the presence of an interface. Some have thought of air:water or of Oparin's (1938) liquid:liquid interfaces; these fit well into the ideas of traditional biochemistry. Goldschmidt (1952) made the valuable suggestion that the faces and edges of mineral crystals could have played an important part in the concentration and collection of uncommon molecules from the environment and their arrangement in ordered and reactive groups. In his paper he wrote of such crystals as quartz, mica, clay minerals, apatite, and snow; but in conversation he spoke of the more reactive crystals that contain elements such as Cu, Fe, Mn, and V. Working from this idea I suggested (Pirie, 1948) that at least an analogue for an organism would be made if the synthesis of a water-holding substance on a crystal depended on the leaching out of the active element and, by the retention of water, promoted that leaching. If such a suggestion is taken seriously, we can dismiss from consideration tables of the relative abundance of the chemical elements. It matters little how rare the regions are so long as they exist, for they serve only to initiate the process. Once begun, other activities, depending perhaps on other interfaces, would accrete to the system and, as Haldane (1954b) suggests, independent subvital units might cooperate.

In a quodlibet or parable such as that there is no necessity to limit consideration to carbon compounds. Carbon compounds of various sorts probably were present at the beginning, and they may well have been among the compounds that participated. Life, considered throughout its history, can be likened to a pair of cones placed apex to apex. The base is the group of eobionts, dependent on a very wide range of different chemical actions and energy sources that modify the original environment on the earth and, in the course of time, compete for sites, energy, substrates, and so on. The narrowing of the cone represents the elimination of many forms, partly by exhaustion of their substrates, partly by competition. In this way biochemical complexity and efficiency would build up until, at the common apices, there would be a few varieties comparable to present-day autotrophic bacteria. Half the present-day biomass is bacterial; this picture assumes that at that stage, well before the beginning of the fossil record, all of it was. Once this evolution and selection for biochemical efficiency has taken place, it is very difficult to see past it to the more diverse biochemical state by which it was preceded. Using the analogy

of the cones, it is hard to see through the constriction to the broadening cone below. The only clues, as I have argued elsewhere (Pirie, 1957), may come from some surviving oddities of metabolism in ancient species, and from some elementary associations in sedimentary deposits.

From this apex of biochemical capacity the upper cone broadens, representing the evolution of morphological complexity. It is of this that the fossil record gives evidence, and it is this that is accompanied by the biochemical degeneration that is such a common feature of evolution (Lwoff, 1943). Morphologic elaboration permits biochemical inadequacy; it may even encourage it. An organism, at the primitive stage at which it is a simple bag that contains enzymes, depends on metabolites diffusing to it and must make everything it needs from whatever materials may come to hand. This puts a premium on biochemical efficiency and adaptability. An organism such as that needs the unspecialized genius of a successful Robinson Crusoe. Along with morphologic and mechanical evolution goes a greater independence from the environment, because the organism can increasingly recognize favorable or noxious conditions and arrange to enjoy or avoid them. For a mechanically highly evolved organism, biochemical expertness loses some of its survival value. We see this in the great synthetic capacity of the sessile plants, as compared to the more generally motile animals and, to some extent, in the distribution of such things as vitamin requirement among the animals.

Many other assumptions could have been discussed usefully, but one more must suffice. It is commonly assumed that the occurrence of optical activity is peculiarly significant in biology, and that its initial appearance presents difficulties. However, the possibility of optical isomerism arises of necessity by simple geometry at a certain level of chemical complexity, and the probability that in a synthesis there will be a preponderance of one isomer increases as the amount of material that is synthesized diminishes, as Karl Pearson pointed out long ago. Once there is a bias, selection would favor any organism that used only one isomer. Although there is nothing surprising in optical activity in organisms, it might still be peculiar to them. There have been, however, several recent papers on spontaneous resolutions (Powell, 1952), and naturally occurring mineral crystals will supply regions of selective absorption. The argument that the appearance of optical activity in oil, after it has percolated through great thicknesses of assymetric rock crystals, is evidence for its biological origin is therefore suspect, however valid the conclusion may be.

All discussion depends on assumptions. My thesis here is not that assumptions should not be made, but that we should be aware that they are being made, and that we should consider whether they are needed for the real purpose of the discussion. Even when they are not needed in theory, they still may be useful; some people, whether engaged in observation or argument, need the extra stability that seems to come from a body of assumption. So long as the observations are sound, the status of the assumptions is secondary. It is better to have a productive scientist with a questionable philosophy than one who observes little, but whose philosophy is impeccable.

A critical examination of some commonly made assumptions, however, shows how difficult it is to formulate the problem of spontaneous generation in a

manner that poses questions that can be answered in the laboratory. We do not know what we are trying to generate, or from what. It is certain that further research will demonstrate the existence of a vast range of types of molecules that might be formed from different elements in the different possible environments and that could then interact. From among these reactions it will be possible to map routes along which analogues for life could have proceeded. This route mapping will be slow and intricate and, by the time we are well embarked on it, there may seem to have been some loss in the spontaneity of spontaneous generation.

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